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PCT/GB2005/050002

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The Patent Office

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Cardiff Road
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1. Your reference — MEDY / P29595GB

2. Patent application number *(The Patent Office will fill in this part)* — 0400290.3 — 0 8 JAN 2004

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Medivir AB
Lunastigen 7
141 44 Huddinge
Sweden

Patents ADP number *(if you know it)* 8695322001

If the applicant is a corporate body, give the country/state of its incorporation — Sweden

4. Title of the invention

dUTPase inhibitors

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

ERIC POTTER CLARKSON
PARK VIEW HOUSE
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NOTTINGHAM
NG1 5DD

Patents ADP number *(if you know it)* **1305010**

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) *any applicant named in part 3 is not an inventor; or*
b) *there is an inventor who is not named as an applicant, or*
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Description	59
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Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	YES ✓
Request for preliminary examination and search *(Patents Form 9/77)*	YES ✓
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Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Eric Potter Clarkson.

Signature
ERIC POTTER CLARKSON

Date
7 January 2004

12. Name and daytime telephone number of person to contact in the United Kingdom **0115 9552211**

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dUTPase inhibitors

Field of the Invention

The present invention relates to pharmaceuticals active against parasite dUTPase and methods for treating parasitical infections, especially malaria, by administering such compounds.

Technical Background

Deoxyuridine triphosphate nucleotidohydrolase (dUTPase) is an ubiquitous enzyme which hydrolyzes deoxyuridine triphosphate (dUTP) to deoxyuridine monophosphate (dUMP) and pyrophosphate. This reaction is thought to occur primarily to limit pools of intracellular dUTP in order to prevent significant uridine incorporation into DNA during replication and repair. A second role of dUTPase is to provide substrate (dUMP) for the de novo synthesis of thymidylate.

Two groups of researchers, McIntosh et al., PNAS, 89:8020-8024 (1992) and Strahler et al., PNAS, 90:4991-4995 (1993), have reportedly isolated the human dUTPase enzyme and characterized the enzyme by its cDNA and amino acid sequences.

McIntosh reported a cDNA of 526 base pairs containing an ORF which encoded a protein of 141 amino acids and a 3f flanking sequence following the ORF. Strahler reported the identical cDNA and amino acid sequence as did McIntosh, with the exception of two additional bases at the 51 end of the cDNA and a longer 31 flanking sequence. The human dUTPase reported by both groups was found to have a high degree of homology with dUTPase from other organisms including that from yeasts, bacteria and viruses. Strahler further reported that human dUTPase exists in both, phosphorylated and a non-phosphorylated forms.

International patent application no WO97/36916 discloses the sequence of nuclear and mitochondrial isoforms of dUTPase.

In both prokaryotic and eukaryotic cell systems, dUTPase has been clearly shown to be an essential enzyme, without which the cell will die. Lack of dUTPase leads to elevated cellular dUTP pools, resulting in an increased misincorporation of uridine into DNA. In addition to prokaryotes and eukaryotes, a number of viruses, such as herpes simplex, are known to encode a dUTPase function.

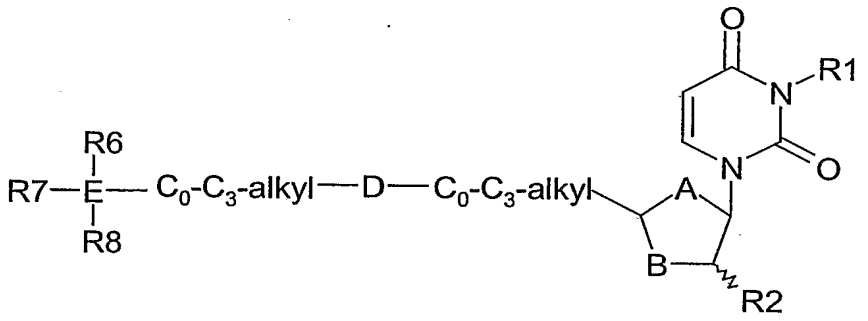
International patent application no WO95/15332 proposes a range of uridine di- and triphosphate analogues in which the oxygen atoms between phosphate groups are replaced with methylene, secondary amine or tertiary amine, and/or oxo functions on the phosphate are replaced with sulphur. These compounds are postulated as cytostatics for use against rapidly growing cancer cells and/or antivirals against herpes.

The present inventors have established that the substrate specificity of the dUTPases of certain protozoal and bacterial parasites of man differ from the corresponding human cellular and mitochondrial enzymes to such an extent that a specific set of inhibitor compounds can be prepared which selectively inhibit the parasite dUTPase without substantially inhibiting the human counterparts. Examples of such parasites include Plasmodium species especially P.falciparum responsible for malaria, Mycobacterial species, especially M tuberculosum responsible for tuberculosis and Leishmania spp.

Hidalgo-Zarco and González-Pacanowska Current Protein and Peptide Science, 2001, 2, 389-397 describe the isolation and characterisation of trypanosomal dUTPases. Competitive inhibition of Leishmania dUTPase was shown by the triphosphate substrate analogue α-β-imido-dUTP, whereas no inhibition of that parasite was apparent in the case of 5'-O-(4-4'-dimethoxytrityl)-2'-deoxyuridine.

Brief description of the Invention

In accordance with a first aspect of the invention there are provided novel compounds of the formula I



I

where

A is O, S or CH_2;

B is O, S or CHR^3;

R^1 is H, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl or a 5 or 6 membered, saturated or unsaturated ring containing 0 to 3 heteroatoms selected from N, O and S, any of which is optionally substituted with R^4;

R^2 is H or F;

R^3 is H, F, OH, NH_2 or a pharmaceutically acceptable ester, amide or ether thereof; or

R^2 and R^3 together form a chemical bond;

D is –NHCO-, -CONH-, -O-, -C(=O)-, -CH=CH, -C≡C-, -NR^5-,

R^4 is hydrogen, halo, cyano, amino, nitro, carboxy, carbamoyl, hydroxy, oxo, C_1-C_5 alkyl, C_1-C_5 haloalkyl, C_1-C_5 alkyloxy, C_1-C_5 alkanoyl, C_1-C_5 alkanoyloxy, C_1-C_5 alkylthio, -N(C_0-C_3-alkyl)$_2$, hydroxymethyl, aminomethyl, carboxymethyl; -SO_2N(C_0-C_3-alkyl), -SO_2C_1-C_5-alkyl;

R^5 is H, C_1-C_3-alkyl, C_1-C_3-alkanoyl;

E is Si or C;

R^6, R^7 and R^8 are independently selected from C_1-C_8 alkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, or a stable monocyclic, bicyclic or tricyclic ring system which is saturated or unsaturated in which each ring has 0 to 3 heteroatoms

selected from N, O and S, wherein any R^6, R^7 and R^8 group may be optionally substituted with R^4;
with the proviso that if R^3 is H, OH, F, NH_2 or a bond, then at least one of R^6, R^7 and/or R^8 comprises an unsaturated ring, and the further proviso that the compound is not 5'-O-(4-4'-dimethoxytrityl)-2'-deoxyuridine;
and pharmaceutically acceptable salts thereof.

Conveniently, A is –O- and B is $–CHR^3-$ thus defining a 2'-deoxyribose analogues.

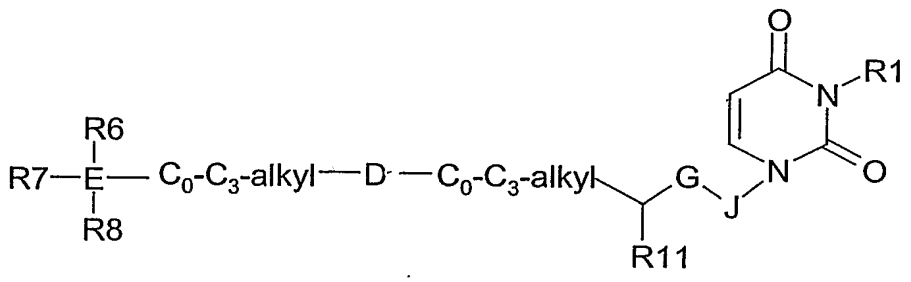
Alternative preferred variants include those where A is –O- and B is -O-, or –S-, thus defining a dioxolane or especially an oxathiolane derivative.

Other preferred variants include those wherein R^2 and R^3 form a chemical bond and A is –O-, thus defining a 2'3'-dideoxy, didehydroribose derivative or R^2 and R^3 are H, thus defining a 2',3'-dideoxyribose derivative.

Still further preferred variants include those wherein R^2 and R^3 form a chemical bond and A is $–CH_2-$, thus defining a 2-cyclopentene derivative or those wherein R^2 and R^3 are H, thus defining a cyclopentane derivative.

It is currently preferred that R^3 is H, OH or F. An alternative R^3 is a lipophilic ester such as straight or branched chain alkyl or benzyl ester or an ether such as straight or branched chain alkyl or benzyl ether or alkylated silyl function.

A further aspect of the invention provides acyclic compounds of the formula II:



II

wherein R^1 is H, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl or a 5 or 6 membered, saturated or unsaturated ring containing 0 to 3 heteroatoms selected from N, O and S, any of which is optionally substituted with R^4;

D is –NHCO-, -CONH-, -O-, -C(=O)-, -CH=CH, -C≡C-, -NR^5-,

R^4 is hydrogen, halo, cyano, amino, nitro, carboxy, carbamoyl, hydroxy, oxo, C_1-C_5 alkyl, C_1-C_5 haloalkyl, C_1-C_5 alkyloxy, C_1-C_5 alkanoyl, C_1-C_5 alkanoyloxy, C_1-C_5 alkylthio, -N(C_0-C_3-alkyl)$_2$, hydroxymethyl, aminomethyl, carboxymethyl; -SO_2N(C_0-C_3-alkyl), -SO_2C_1-C_5-alkyl;

R^5 is H, C_1-C_3-alkyl, C_1-C_3-alkanoyl;

E is Si or C;

R^6, R^7 and R^8 are independently selected from C_1-C_8 alkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, or a stable monocyclic, bicyclic or tricyclic ring system which is saturated or unsaturated in which wherein each ring has 0 to 3 heteroatoms selected from N, O and S, and wherein any of which R^6, R^7 and R^8 groups are optionally substituted with R^4;

G is –O-, -S-, -CHR^{10}-, -C(=O)-;

J is CH_2, or when G is CHR^{10} may also be –O- or –NH-;

R^{10} is H, CH_3, CH_2NH_2, CH_2OH, OH;

R^{11} is H, F, CH_3, CH_2NH_2, CH_2OH, $CH(OH)CH_3$, $CH(NH_2)CH_3$, or a pharmaceutically acceptable ether, amide or ester thereof; or

R^{10} and R^{11} together define an olefinic bond, or together form a –CH_2- group, thereby defining a *cis* or *trans* cyclopropyl group;

with the proviso that if R^{11} is other than an ether, amide or ester, then at least one of R^6, R^7 and/or R^8 comprises an unsaturated ring:

and pharmaceutically acceptable salts thereof.

Preferably G is –O-, that is an N-1 methyloxymethyluridine derivative or $-CH_2-$, that is an N-1-alkyl derivative. Additional preferred variants at this position include wherein G is $-CH(CH_2OH)-$ or wherein R^{10} and R^{11} define an olefinic bond or a cyclopropyl group.

Preferred R^{11} groups include H and CH_2OH or lipophilic ethers or esters thereof such as a straight or branched chain alkyl or benzyl ester or an ether such as straight or branched chain alkyl or benzyl ether or an alkylated silyl function.

Certain preferments of the invention tend to be applicable to both formula I and II. For example R^1 is preferably a small substituent, most preferably H.

Favoured C_0-C_3-alkyl-D-C_0-C_3-alkyl configurations include aminomethyl, aminoethyl and aminopropyl, methylaminomethyl, methylaminoethyl, ethylaminomethyl, -(N-methyl)aminomethyl, -(N-methyl)aminoethyl, -(N-methyl)aminopropyl and methyl-(N-methyl)aminomethyl. Currently the most preferred is –aminomethyl-. The order of the hetero atom and alkylene moieties in the indicated groups as used herein corresponds to the configuration of Figure I or II as depicted above, that is "aminomethyl" has the nitrogen atom adjacent E.

Particularly preferred C_0-C_3-alkyl-D-C_0-C_3-alkyl configurations include –O-, oxymethyl, oxyethyl, oxypropyl methyloxymetyl and methyloxyethyl. Currently the most preferred is –oxymethyl-.

Preferably at least one of R^6, R^7 and/or R^8 has an aromatic nature, although this tends to be less important if R^{11} or R^3 has a lipophilic nature. Conveniently two of R^6, R^7 and/or R^8 have an aromatic nature, the invention even embraces compounds wherein all three have an aromatic nature.

Convenient values for R^6, R^7 and/or R^8 include heterocyles such as furyl, thienyl, pyranyl, pyrrolyl, pyrrolinyl, pyrrolidinyl, pyrazolyl, pyrazolinyl, pyrazolidinyl, imidazolyl, imidazolinyl, imidazolidinyl, pyridyl, piperidinyl, pyrazinyl, piperazinyl, pyrimidinyl, pyridazinyl, oxazolyl, oxazolidinyl, isoxazolyl, isoxazolidinyl, morpholinyl, thiazolyl, thiazolidinyl, isothiazolyl, isothiazolidinyl, especially pyridyl, and carbocycles such as cycloalkyl, cycloalkenyl and especially phenyl. Alternative values for R^6, R^7 and/or R^8 include straight or branched alkyl, including methyl, ethyl, i-propyl and t-butyl.

Favoured $R^6(R^7)(R^8)$-E- configurations include $-C(Ph)_3$ (trityl), $-CH(Ph)_2$, $-CH_2Ph$, $-Si(t\text{-}Bu)(CH_3)_2$, $-Si(Ph)_2(t\text{-}Bu)$, 1,1-bis(4-methylphenyl)-1'-pyrenylmethyl.

The compounds of the invention include a number of chiral centres, and the invention extends to include racemates, enantiomers and stereoisomers at each of these centres. For example the ring carbon attached to the uracil N1 in Formula I may be in the alpha (down) or preferably the beta (up) configuration. R_2 as F in Formula I may be in the ribo (down) position although it is currently preferred to have the arabino (up) position. It is currently preferred that the ring carbon intermediate A and B in Formula I projects the adjacent C_0-C_3 alkyl in the beta configuration.

Similarly, the invention extends to all stereochemistries around G (as CHR^{10}, where R^{10} is other than H) and R^{11} in Figure II, including cis and trans cyclopropyl for R^{10} and R^{11}.

Compounds of the invention are generally at least 80% preferably at least 90% such as 97% stereoisometrically pure at chiral centres.

Additional aspects of the invention include a pharmaceutical composition comprising a compound of the formula I or II in conjunction with a pharmaceutically acceptable carrier or diluent therefore. The invention further provides a method for the treatment or prophylaxis of parasite infections, such as malaria, in man or a zoonose vector comprising the administration of an effective amount of a compound of the formula I but including the compound appearing in the proviso or II to a patient in need thereof, or to the vector.

While it is possible for the active agent to be administered alone, it is preferable to present it as part of a pharmaceutical formulation. Such a formulation will comprise the above defined active agent together with one or more acceptable carriers or excipients and optionally other therapeutic ingredients. The carrier(s) must be acceptable in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient.

The formulations include those suitable for rectal, nasal, topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration, but preferably the formulation is an orally administered formulation. The formulations may conveniently be presented in unit dosage form, e.g. tablets and sustained release capsules, and may be prepared by any methods well known in the art of pharmacy.

Such methods include the step of bringing into association the above defined active agent with the carrier. In general, the formulations are prepared by uniformly and intimately bringing into association the active agent with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product. The invention extends to methods for preparing a pharmaceutical composition comprising bringing a compound of Formula I or its pharmaceutically acceptable salt in conjunction or

association with a pharmaceutically acceptable carrier or vehicle. If the manufacture of pharmaceutical formulations involves intimate mixing of pharmaceutical excipients and the active ingredient in salt form, then it is often preferred to use excipients which are non-basic in nature, i.e. either acidic or neutral.

Formulations for oral administration in the present invention may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active agent; as a powder or granules; as a solution or a suspension of the active agent in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water in oil liquid emulsion and as a bolus etc.

With regard to compositions for oral administration (e.g. tablets and capsules), the term suitable carrier includes vehicles such as common excipients e.g. binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, polyvinylpyrrolidone (Povidone), methylcellulose, ethylcellulose, sodium carboxymethylcellulose, hydroxypropylmethylcellulose, sucrose and starch; fillers and carriers, for example corn starch, gelatin, lactose, sucrose, microcrystalline cellulose, kaolin, mannitol, dicalcium phosphate, sodium chloride and alginic acid; and lubricants such as magnesium stearate, sodium stearate and other metallic stearates, stearic acid, glycerol stearate, silicone fluid, talc waxes, oils and colloidal silica. Flavouring agents such as peppermint, oil of wintergreen, cherry flavouring or the like can also be used. It may be desirable to add a colouring agent to make the dosage form readily identifiable. Tablets may also be coated by methods well known in the art. A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active agent in a free flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, preservative, surface-active or dispersing agent. Moulded

tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may be optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active agent.

Other formulations suitable for oral administration include lozenges comprising the active agent in a flavoured base, usually sucrose and acacia or tragacanth; pastilles comprising the active agent in an inert base such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active agent in a suitable liquid carrier.

Dosages are set in the conventional manner to take into account the severity of the disease, the susceptibility of the parasite strain, the size and metabolic health of the patient, the mode and form of administration, concomitant medication and other relevant factors. The compounds of the invention may be administered at a daily dose generally in the range 0.1 to 200 mg/kg/day, advantageously, 0.5 to 100 mg/kg/day, more preferably 10 to 50mg/kg/day, such as 10 to 25 mg/kg/day. A typical dosage rate for a normal adult will be around 50 to 500 mg, for example 300 mg, once or twice per day.

The compounds of formula I and formula II can form salts which form an additional aspect of the invention. Appropriate pharmaceutically acceptable salts of the compounds of formula I include salts of organic acids, especially carboxylic acids, including but not limited to acetate, trifluoroacetate, lactate, gluconate, citrate, tartrate, maleate, malate, pantothenate, isethionate, adipate, alginate, aspartate, benzoate, butyrate, digluconate, cyclopentanate, glucoheptanate, glycerophosphate, oxalate, heptanoate, hexanoate, fumarate, nicotinate, palmoate, pectinate, 3-phenylpropionate, picrate, pivalate, proprionate, tartrate, lactobionate, pivolate, camphorate, undecanoate and succinate, organic sulphonic acids such as methanesulphonate, ethanesulphonate, 2-hydroxyethane sulphonate, camphorsulphonate, 2-napthalenesulphonate,

benzenesulphonate, p-chlorobenzenesulphonate and p-toluenesulphonate; and inorganic acids such as hydrochloride, hydrobromide, hydroiodide, sulphate, bisulphate, hemisulphate, thiocyanate, persulphate, phosphoric and sulphonic acids.

Examples of monocyclic rings for R^1 include heterocycles such as furyl, thienyl, pyranyl, pyrrolyl, pyrrolinyl, pyrrolidinyl, pyrazolyl, pyrazolinyl, pyrazolidinyl, imidazolyl, imidazolinyl, imidazolidinyl, pyridyl, piperidinyl, pyrazinyl, piperazinyl, pyrimidinyl, pyridazinyl, oxazolyl, oxazolidinyl, isoxazolyl, isoxazolidinyl, morpholinyl, thiazolyl, thiazolidinyl, isothiazolyl, isothiazolidinyl, especially pyridyl, and carbocycles such as cycloalkyl, cycloalkenyl and phenyl.

Examples of monocyclic, bicyclic or tricyclic rings for R^6, R^7 and/or R^8 include heterocycles such as furyl, thienyl, pyranyl, pyrrolyl, pyrrolinyl, pyrrolidinyl, pyrazolyl, pyrazolinyl, pyrazolidinyl, imidazolyl, imidazolinyl, imidazolidinyl, pyridyl, piperidinyl, pyrazinyl, piperazinyl, pyrimidinyl, pyridazinyl, oxazolyl, oxazolidinyl, isoxazolyl, isoxazolidinyl, morpholinyl, thiazolyl, thiazolidinyl, isothiazolyl, isothiazolidinyl, thiadiazolyl, tetrazolyl, triazolyl, and the like or bicyclic rings especially of the above fused to a phenyl ring such as indolyl, quinolyl quinolinyl, isoquinolinyl, benzimidazolyl, benzothiazolyl, benzoxazolyl, benzotriazolyl, benzofuryl, benzothienyl etc. Additional rings include xanthenyl (such as 9-xanthenyl, 9-alkylxanthenyl, 9-(9-alkyl)xanthenyl, 9-phenylxanthenyl, 9-(9-phenyl)xanthenyl, 9-heteroarylxanthenyl, 9-(9-heteroaryl)xanthenyl), dibenzosuberyl, 5-dibenzosuberyl, fluorenyl (such as 5-fluorenyl, 5-(5-alkyl)fluorenyl, 5-(5-phenyl)fluorenyl, 5-(5-heteroaryl)fluorenyl) and the like.

Examples of carbocycles for R^6, R^7 and/or R^8 include monocyclic rings such as phenyl, cyclohexenyl, cyclopentenyl, cyclohexanyl, cyclopentanyl, bicyclic rings such as indanyl, napthyl, and tricyclic rings such as adamantyl, and the like.

The carbo or heterocyclic ring may be bonded via a carbon or via a hetero atom, typically a nitrogen atom, such as N-piperidyl, N-morpholinyl etc. Other examples of such ring systems may also be found in J. Fletcher, O. Dermer, R. Fox, Nomenclature of Organic Compounds, pp. 20-63 (1974).

The term "C_1-C_5 alkyl" includes such groups as methyl, ethyl, n-propyl, isopropyl, n-butyl, s-butyl, t-butyl, cyclopropyl, n-pentyl and the like with C_1-C_8 alkyl further including n-hexyl, 3-methylpentyl, and the like.

The term "halo" and "halogen" refer to chloro, bromo, iodo, and especially fluoro.

"C_1-C_5 alkoxy" refers to those groups such as methoxy, ethoxy, propoxy, t-butoxy and the like.

"C_2-C_5 alkenyl" refers to those groups such as vinyl, 1-propen-2-yl, 1-butene-4-yl, 1-pentene-5-yl, 1-butene-1-yl and the like, with C_2-C_8 alkenyl further including hex-3-enyl and the like.

"C_1-C_5 alkylthio" refers to those groups such as methylthio, ethylthio, t-butylthio, and the like.

"C_1-C_5 alkanoyl" refers to groups such as acetyl, propionyl, butyryl and the like.

"C_1-C_5 alkanoyloxy" refers to those groups such as acetoxy, propionoxy, formyloxy, butyryloxy, and the like.

The term "C_2-C_8 alkenoxy" includes groups such as ethenyloxy, propenyloxy, iso-butoxy ethenyl, and the like.

The term "C_2-C5 alkynyl" includes groups such as ethynyl, propynyl, butynyl, pentynyl, and the like with C_2-C_8 alkynl further including hexynyl and the ike .

The term "halo C_1-C_5 alkyl" includes alkyls substituted 1, 2 or 3 times by a halogen including groups such as trifluoromethyl, fluoromethyl, 2-dichloroethyl, 2,2-difluoroethyl, 2,2,2-triflouroethyl, 3,3-difluoropropyl, 1,1-2,2,2 pentafluoroethyl and the like.

The term -C_0-C_3-alkyl- as a bivalent in expressions such as -C_0-C_3-alkyl-D-C_0-C_3-alkyl includes a bond (i.e C_0), methylene (C_1), ethylene (C_2), 1,1-dimethyl-methylene (C_3), propylene (C_3) and the like, with each -C_0-C_3-alkyl- being selected independently.

The term (C_0-C_3-alkyl) in monovalent expressions includes H (ie C_0), Me (C_1), Et (C_2), propyl (C3) with each C_0-C_3-alkyl being selected independently. Accordingly -N (C_0-C_3-alkyl)$_2$ includes –NH_2, - NHMe, NHEt NHPr,-N(Me)$_2$, N(Et)$_2$ etc, -SO_2N(C_0-C_3-alkyl)$_2$, includes -SO_2NH2, -SO_2NHMe, -SO_2N(Me)$_2$ etc

As used herein, "the esters, amides and ethers thereof" refer to the appropriate derivatives of each of the preceding hydroxyl and/or amino groups in the definition. For example the ethers and esters of R^{11} include those of the preceding CH_2OH, $CH(OH)CH_3$ and the amides include those formed by reaction of an amine with either of these groups or by reaction of a carboxylic acid with the indicated -CH_2NH_2 or $CH(NH_2)CH_3$ moieties.

Pharmaceutically acceptable esters include C_1-C_{22} fatty acid esters, such as acetyl, t-butyl or long chain straight or branched unsaturated or omega-6 monounsaturated fatty acids such as palmoyl, stearoyl and the like.

Alternative aryl or heteroaryl esters include benzoyl, pyridylmethyloyl and the like any of which may be substituted with R^4, Preferred pharmaceutically acceptable esters include aliphatic L-amino acid esters such as leucyl, isoleucyl and especially valyl. Additional preferred amino acid esters include the 2-O-AA-C_3-C_{22} fatty acid esters described in WO99 09031, where AA is an aliphatic amino acid ester, especially those derived from L-lactic acid and L-valyl.

Pharmaceutically acceptable ethers include straight or branched chain saturated or omega 6 unsaturated C_1-C_{22} alkyl ethers such as methyl ethers, t-butyl ethers or aryl or heteroaryl ethers such as phenoxy, benzylether, pyridylmethyl ether, any of which may be substituted with R^4.

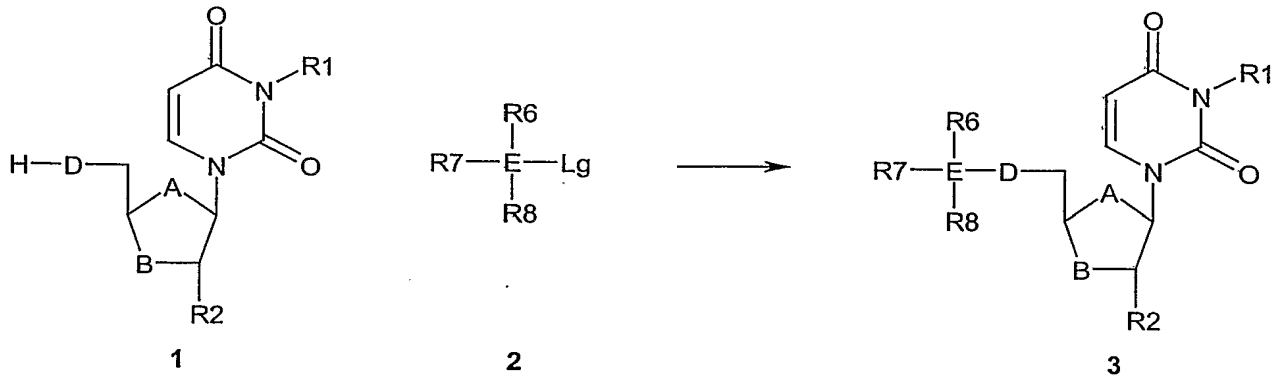
Alternative ethers include alkylated silyl functions such as -Si(C_1-C_5-alkyl)$_3$ such as -Si(t-Bu)(CH_3)$_2$, or -Si(Ph)$_2$(t-Bu), –C(Ph)$_3$ (trityl), -CH(Ph)$_2$, -CH_2Ph,1,1-bis(4-methylphenyl)-1'-pyrenylmethyl and the like.

Pharmaceutically acceptable amides include those derived from C_1-C_{22} branched or straight chain aminoalkyl optionally including 1 to 3 unsaturations, or anilines or benzylamines. Other pharmaceutically acceptable amides of amine functions of R^2 or R^{11} correspond to the esters indicated above.

It is currently preferred that the ester, amide or ether is lipophilic in nature.

Compounds of the invention are typically synthesized as outlined below.

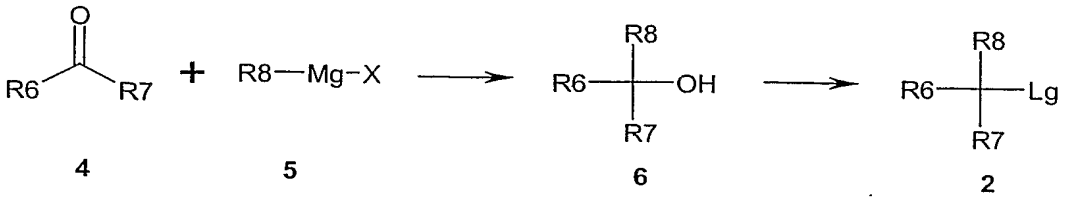
Scheme 1 depicts a method for alkyltion of the 5'-position of a nucleoside or a nucleoside analogue.



1 2 3

Scheme 1

Nucleoside derivative (1) wherein A, B, R^1 and R^2 are as defined above for formula (I) and D is O or NH, can be reacted with an alkylating agent of formula 2 wherein R^6, R^7, R^8 and E are as defined above for formula I and Lg is a leaving group that can be replaced by the nucleophile D, in a solvent like pyridine optionally in the presence of a catalyst such as dimethylaminopyridine or in a solvent like dimethylformamide in the presence of a catalyst like imidazole, to provide 5'-alkylated nucleoside analogues (3). Various alkylating agents (2) are available either commercially or in the literature, se for example Greene, "Protective Groups in Organic Synthesis (John Wiley & Sons, New York, 1981). For example, they can be prepared by transforming the hydroxy group of the corresponding alcohol into a leaving group such as a halide like chloride or bromide by treatment with a halogenating agent such as acetyl bromide or thionyl chloride or the like or they can be transformed into a derivative of sulfonic acid like a mesyl, tosyl, triflate or the like by treatment with for example the anhydride or acid chloride of the desired sulfonic acid derivative. An example of a route to alkylating agents (2) is shown in scheme 2.

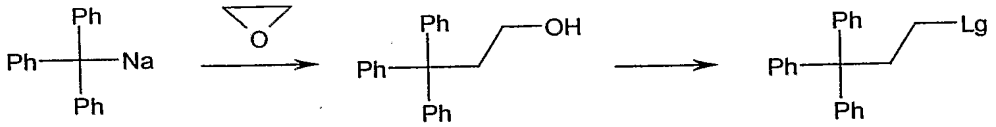


4 5 6 2

Scheme 2

Reaction of an electrophilic carbonyl compound like a keto compound (4) or any carboxylic acid derivative for instance an ester or acid halide, and a suitable nucleophile for example a Grignard reagent (5) or an organolithium reagent, provides alcohol 6. The formed hydroxy group can subsequently be transformed into a leaving group as described above. Examples of the above procedure are described in the literature, se for example Hodges *et al.*, J. Org. Chem. 56, 1991, 449-452, and Jones *et al.*, J. Med. Chem. 33, 1990, 416-419.

Compounds containing a C_1-C_3-alkyl chain, available commercially or in the literature may also be used as alkylating agents in scheme 1. An example of a route to a compound containing a C_2-alkyl chain is shown in scheme 3.



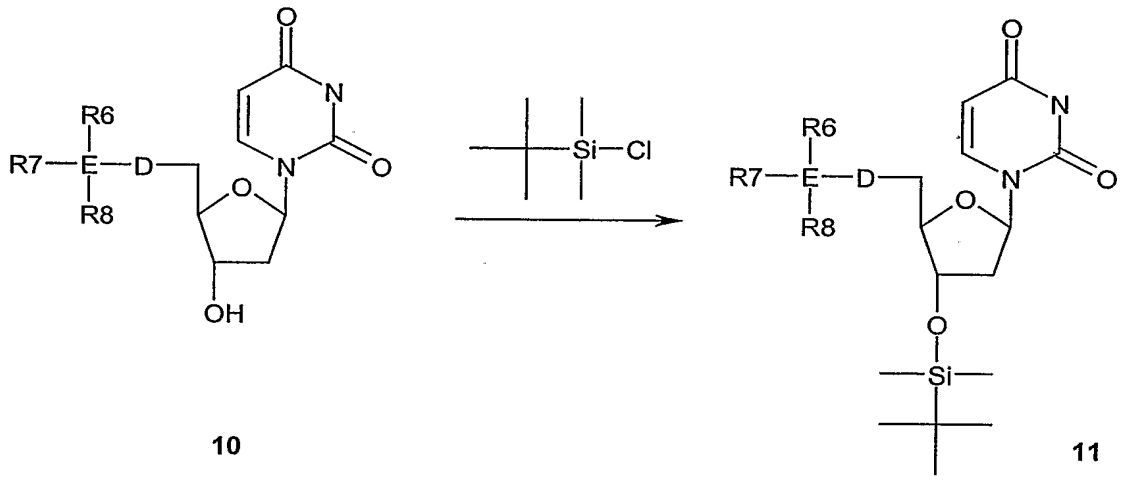
7 8 9

Scheme 3

A reaction performed with triphenylmethyl sodium (7) and ethylene oxide provides alcohol (8). Subsequent transformation of the hydroxy group into a leaving group for example as described above provides alkylating agent (9). Use of any other appropriate electrophilic reagent for example formaldehyde, provides analogues with other length of the C_1-C_3-alkyl chain. Se for example Wooster *et al.*, J. Amer. Chem. Soc., 60, 1938, 1666 and McPhee *et al.*, J. Amer. Chem. Soc. 65, 1943, 2177, 2180. Alternatively, alkylating agents containing a C_1-C_3-alkyl chain may be obtained by reduction of an appropriate acyl derivative to the desired alcohol.

A suitable acylating agent like the acid chloride or anhydride can be used to acylate the amino group of a 5'-aminonucleoside, thus providing compounds according to the general formula I where D is -CONH-.

An example of the introduction of an ether group at the 3'-position of the nucleoside analogue is shown in scheme 4.



10 11

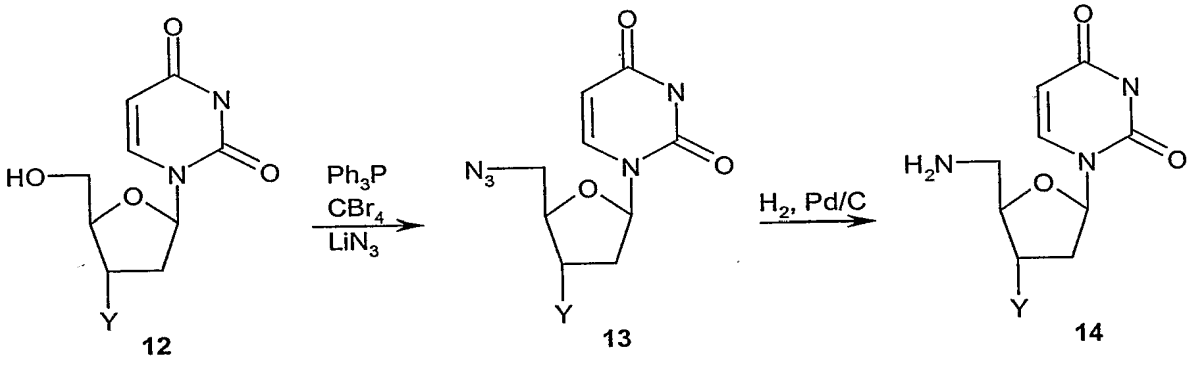
Scheme 4

Treatment of a 5'-substituted nucleoside analogue (10) with a silylating agent for example *tert*-butyldimethylsilyl chloride in a solvent like dimethylformamide in the presence of a catalyst like imidazole, provides 3'-O-silylated derivatives.

Other ether or ester groups can be introduced at the 3'-position by methods known in the art, for example by treating the 3'-OH nucleoside with the desired alkylating or acylating agent optionally in the presence of a suitable base, se for example Greene, "Protective Groups in Organic Synthesis (John Wiley & Sons, New York, 1981).

Nucleoside analogues used in the synthesis of compounds according to the present invention are available either commercially or in the literature or they can be prepared as described herein. For example compound 1 where B is CH_2F, R^1 and R^2 are H and A and D are O *i.e.* FLU (3'fluoro-2',

3'-dideoxyuridine) can be prepared in analogy with the procedure described for FLT (Balzarini *et al.* Biochem. Pharmacol. 37, 2847, 1988). The didehydro derivative d4U (2',3'-didehydro-2',3'-dideoxyuridine) can be prepared in analogy with the procedure described for d4T (2',3'-didehydro-2',3'-dideoxythymidine, Stavudine, Balzarini *et al.*; Mol. Pharmacol. 32, 162, 1987). 5'-aminonucleoside analogues, useful for the preparation of compounds according to the general formula I wherein D is NH or -CONH- can be prepared from the corresponding 5'-alcohols by a displacement-reduction sequence for example as shown in scheme 5.



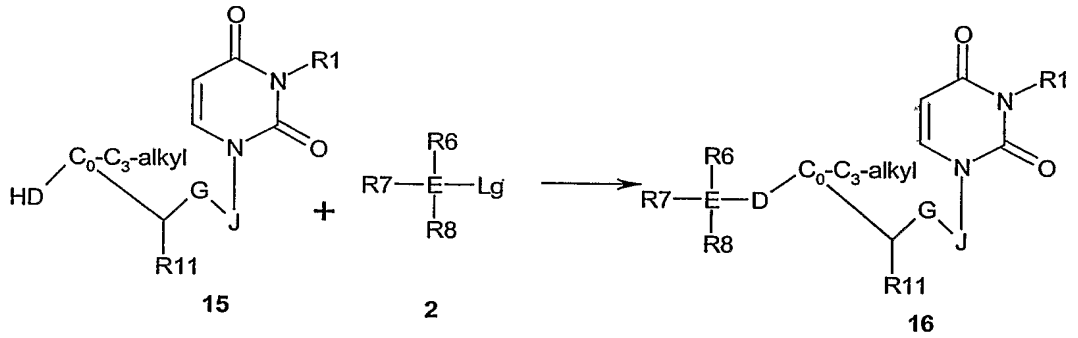
Scheme 5

Nucleoside analogue 12 where Y is F or suitably protected OH can be reacted with triphenylphosphine in a solvent like carbon tetrabromide followed by displacement of triphenylphosphine oxide with azide ion to form 13. Reduction of the azide group for example by catalytic hydrogenation over palladium on carbon, gives the amino derivative 14.

The procedures described in scheme 5 can be also applied to carbocyclic uridine analogues and thiouridine analogues providing compounds useful for the preparation of compounds of the general formula I wherein A is CH_2 and S.

The acyclic and carba acyclic nucleoside analogues of the present invention can be prepared with similar procedures as described above for the cyclic nucleoside analogues.

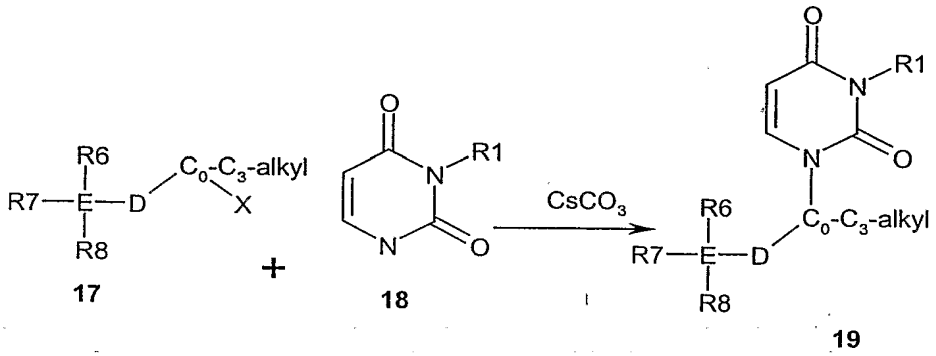
Scheme 6 illustrates the alkylation of acyclic nucleoside analogues.



Scheme 6

Treatment of acyclic nucleoside analogue (15) wherein R^1, R^{11}, G and J are as defined for general formula II and D is O or NH, with the alkylating agent (2) as described for scheme 1, provides alkylated derivative (16). The various alkylating agents discussed above for the cyclic nucleoside analogues, may also be used for the preparation of compounds of the general formula II.

An alternative route to acyclic and carba acyclic nucleoside analogues is shown in scheme 7.

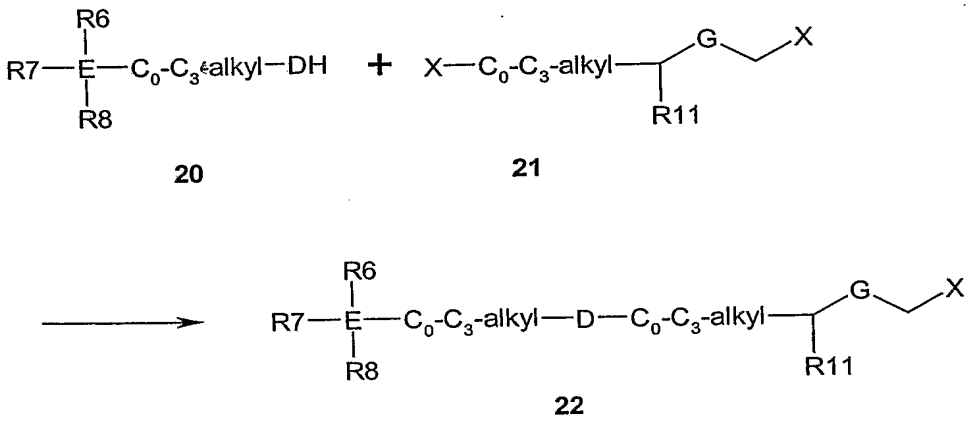


Scheme 7

Alkylation of optionally N-substituted uracil (18) with an alkylating agent (17) wherein R^6, R^7, R^8 and E are as defined above, D is O or NH and X is a leaving group such as chloride or bromide, in the presence of a base

such as cesium carbonate in a solvent like dimethylformamide, provides 19.

Various acyclic side chains may be prepared as depicted in scheme 8.



Scheme 8

Reaction of a nucleophilic compound (20) where R^6, R^7, R^8 and E are as defined above and D is O or NH, in a displacement reaction with an alkylating agent (21) where R^{11} and G are as defined above and X is a leaving group such as a halide like chloride or bromide in a solvent like dimethylformamide in the presence of an appropriate base such as sodium hydride or a carbonate provides acyclic side chains that subsequently can be coupled to the desired uracil derivative as described in scheme 7.

Acyclic nucleoside analogues used for the preparation of compounds according to the present invention are available either commercially or in the literature or they ca be prepared as described herein. For example 1-(2'-hydroxyethoxymethyl)-uracil and 1-(2'-aminoethoxymethyl)-uracil are described by Kelley *et al.* in J. Med. Chem, 1981, 472-475 and J. Med. Chem, 1981,753-756 respectively, the unsaturated compound 1-(hydroxy-2'-butenyl)-uracil can be prepared in analogy with the corresponding thymine derivative as described by Zemlicka *et al.* in J. Med. Chem., 34,

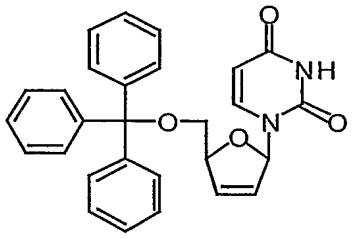
1991, 421-429. Cyclopropyl carbocyclic nucleosides are described for example by Chu *et al.* in J. Org. Chem., 60, 1995, 5236 and Tet. Letters, 37, 1996, 8849-8851. Acyclic uridine analogues useful for the preparation of compounds according to the general formula (II) wherein J is O are described for instance by Harnden *et al.* in J. Chem. Soc. Perkin Trans., 1990, 2175-2183. Compounds wherein J is N can be prepared by condensation of 1-aminouracil with an aproriate aldehyde, as described for 9-aminoalkylguanines by Harnden *et al.* in Tet. Letters, 29, 1988, 5995-5998.

Detailed Description of the Embodiments

Various aspects of the invention will now be described by way of illustration only with reference to the following non-limiting examples.

Example 1

1-(5-Trityloxymethyl-2,5-dihydro-furan-2-yl)-1*H*-pyrimidine-2,4-dione or 5'-*O*-trityl-2',3'-dideoxydidehydrouridine



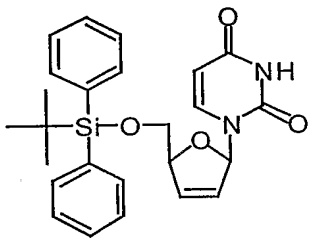
2',3'-Dideoxy-didehydrouridine (0.30g, 1.43mmol) and triphenylmethyl chloride (0.44g, 1.57mmol) was stirred in dry pyridine (10ml) at 50°C overnight under an atmosphere of nitrogen. The reaction mixture was poured into ice-H_2O (30ml) with vigorous stirring and filtered. The precipitate was dissolved in EtOAc (50ml) and the solution was washed with 0.5M HCl (20ml), H_2O (20ml), dried (Na_2SO_4) and reduced *in vacuo* to yield a crude product, which was purified by gradient flash column

chromatography eluting with 0→3% MeOH/$CHCl_3$ to yield the title compound as white crystals (0.37g, 58%).

^1H NMR (300MHz;MeOH): δ 3.56 (2H, m, 5'-H), 5.02 (1H, m, 4'-H), 5.08 (1H,m, 5-H), 5.93 (1H, m, 1'-H), 6.40 (1H, m, 2'-H), 7.09 (1H, m, 3'-H), 7.30-7.44 (15H, m, Ph-H), 7.87 (1H, d, J = 8.1 Hz, 6-H);
^{13}C NMR (75MHz;MeOH): δ 64.82 (5'-CH_2), 86.38 (1'-CH), 87.84 (a), 90.04 (4'-CH), 102.70 (5-CH), 126.79 (Ph-CH), 127.79 (2'-CH), 128.41 (Ph-CH), 129.20 (Ph-CH), 134.89 (3'-CH), 141.79 (6-CH), 143.49 (Ph-C), 151.04 (2-C), 159.95 (4-C);
MS (Cl/NH_3., *m/z*); 470.2 ($M+NH_4^+$, 100%), 453.1 ($M+H^+$, 20%),;
HRMS (ES+ve., M+H): Calculated for $C_{28}H_{24}N_2O_4$, requires 453.1814, found 453.1807.
IR_{vmax}/cm^{-1} (KBr): 714 and 756 (Aromatic, monosubstituted), 1681.0 (C=O) and 1692.3 (C=O);
Mp: 68°C
R_f (10% MeOH / $CHCl_3$): 0.30;

Example 2

1-[5-(*tert*-Butyl-diphenyl-silanyloxymethyl)-2,5-dihydro-furan-2-yl]-1*H*-pyrimidine-2,4-dione or 5'-*O*-*tert*-butyldiphenylsilyl-2',3'-dideoxydidehydrouridine

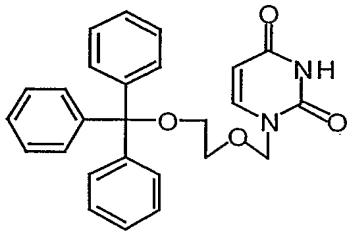


2'3'-dideoxy-didehydrouridine (0.30g, 1.43mmol) in dry DMF (10ml) were added drop-wise under an atmosphere of nitrogen, with ice bath cooling, to a stirred solution of *tert*-butyldiphenyllsilylchloride (0.41ml, 1.57mmol) and imidazole (0.21g, 3.14mmol) in dry DMF (10ml). The mixture was allowed to warm to room temperature and stirred overnight. H_2O (10ml)

was added and the mixture was extracted with $CHCl_3$ (2 x 30ml). The combined extracts were washed with saturated aqueous $NaHCO_3$ solution (10ml) and H_2O (10ml), dried (Na_2SO_4) and reduced *in vacuo* to obtain a crude product, which was purified by gradient flash column chromatography eluting with 0→3% MeOH/$CHCl_3$ to yield the title compound as colourless viscous oil (0.46g, 73%).

^{1}H NMR(300MHz;MeOH): δ 1.15 [9H, s, C(C*H*$_3$)$_3$], 3.95 (1H, dd, *J* = 2.9, 11.7 Hz, 5'-H), 4.06 (1H, dd, *J* = 2.9, 11.7 Hz, 5'-H), 4.97 (1H, m, 4'-H), 5.26 (1H, d, *J* = 8.1 Hz, 5-H), 5.58 (1H, m, 1'-H), 6.38 (1H, m, 2'-H), 7.10 (1H, m, 3'-H), 7.34-7.55 (6H, m, Ph-CH), 7.66-7.81 (5H, m, Ph-H and 6-H).
^{13}C NMR(75MHz;MeOH): δ 19.81 [*C*(CH$_3$)$_3$], 26.99 and 27.42 [C(*C*H$_3$)$_3$], 65.40 (5'-CH$_2$), 87.56 (1'-CH), 90.06 (4'-CH), 102.96 (5-CH), 126.96 (2'-CH), 128.41 (Ph-CH), 128.32 (Ph-CH), 128.12 (Ph-CH), 130.03 (Ph-CH), 130.47 (Ph-CH), 130.59 (Ph-CH), 132.78 (Ph-C), 133.46 (Ph-C), 134.99 (3'-CH), 135.25 (Ph-CH), 135.79 (Ph-CH) 135.99 (Ph-CH), 141.20 (6-CH), 150.99 (2-C), 163.45 (4-C);
MS (CI/NH_3., *m/z*); 449.1 ($M+H^+$, 50%), 466.2 ($M+NH_4^+$, 100%);
HRMS (ES+ve., $M+H^+$): Calculated for $C_{25}H_{28}N_2O_4Si$, requires 449.1896, found 449.1894.
IR_{vmax}/cm^{-1} (film): 1697.3 (C=O).
R_f (10% MeOH / $CHCl_3$): 0.73.

Example 3
1-(2-Trityloxy-ethoxymethyl)-1*H*-pyrimidine-2,4-dione

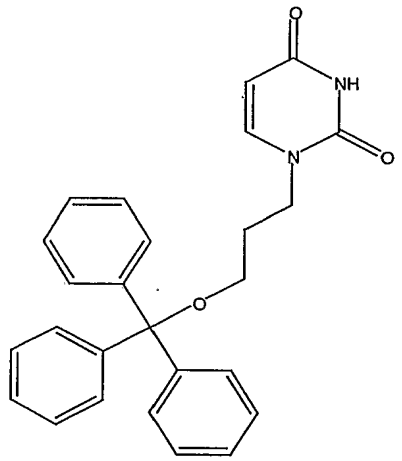


1-(2-hydroxy-ethoxymethyl)uracil (0.50g, 2.69mmol) and triphenylmethyl chloride (0.82g, 2.96mmol) were stirred in dry pyridine (20ml) overnight at 50°C under an atmosphere of nitrogen. H_2O (10ml) was added and the mixture was extracted with $CHCl_3$ (2 x 50ml), dried (Na_2SO_4) and reduced *in vacuo* to obtain a crude product, which was purified by column chromatography eluting with 0→3% MeOH/$CHCl_3$ to obtain the title compound as a white solid (0.23g, 20%).

^{1}H NMR(300MHz;$CDCl_3$): δ 3.32 (2H, 4'-H), 3.78 (2H, m, 5'-H), 5.29 (2H, s, 1'-H), 5.82 (1H, d, *J* = 7.9 Hz, 5-H), 7.29-7.51 (16H, m, Ph-H and 6-H);
^{13}C NMR(75MHz;$CDCl_3$): δ 63.32 (5'-CH_2), 69.70 (4'-CH_2), 77.38 (1'-CH_2), 103.58 (5-CH), 87.19 ($Ph_3\underline{C}$), 127.55 (Ph-CH), 128.31 (Ph-CH), 129.08 (Ph-CH), 143.25 (Ph-CH), 143.52 (6-CH), 151.43 (2-C), 163.81 (4-C);
MS (ES+ve., *m/z*): 451.1 ($M+Na^+$, 100%);
HRMS (ES+ve., $M+Na^+$): Calculated for $C_{26}H_{24}N_2O_4$, requires 451.1634, found 451.1626.
IR_{vmax}/cm^{-1} (KBr): 703.2 and 760.0 (Aromatic-monosubsituted), 1673.4 (C=O), 1701.8 (C=O), 3021.1 (Aromatic, C-H stretching).
Mp: 145-148°C.
R_f (10% MeOH / $CHCl_3$): 0.71.

Example 4

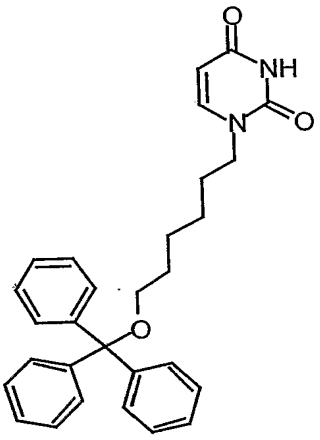
1-[3-(Triphenyl-methanoxy)-propyl]-1H-pyrimidine-2,4-dione or 1-(3'-trityloxylpropyl)uracil



A mixture of 3-bromo-1-*O*-trityl propanol (400 mg, 1.05 mmol), cesium carbonate (385 mg, 1.05 mmol) and uracil (129 mg, 1.15 mmol) in DMF (10 ml) was warmed to 40 °C under atmosphere of nitrogen. After 4 hours the TLC showed the disappearance of the starting material, then water (10 ml) was added into the suspension, and extracted with EtOAc (3 × 15 ml). The organic layer was washed with brine, dried and the solvent was removed under reduced pressure to give a crude residue purified by flash chromatography to afford the desired compound as a white solid (134 mg, 31%).

^{1}H-NMR (300 MHz; $CDCl_3$) δ 2.08 (2H, m, 2'-H), 3.22 (2H, t, *J*=5.67 Hz,1'-H), 4.02 (2H, t, *J*=6.58 Hz, 3'-H), 5.47 (1H, dd, *J*=2.37, 7.87 Hz, 5-H), 6.95 (1H, d, *J*=7.87 Hz, 6-H), 7.22-7.50 (15H, m, Ph-H), 8.10 (1H, bs, 3-NH).

Example 5
1-(4'-trityloxyhexyl)uracil



A solution of uracil (0.066 g, 0.59 mmol) and caesium carbonate (0.135 g, 0.41 mmol) in dry DMF (4 mL) was stirred at room temperature under nitrogen for 10 min. 6-Bromo-1-*O*-tritylhexanol (0.170 g, 0.40 mmol) in dry DMF (1 mL) was added drop-wise *via* a syringe. The reaction mixture was stirred at 50°C for 24 h and then left to cool down. Water (10 mL) was added and the solution was extracted with EtOAc (3x10 mL). The organic extracts were pooled, washed with brine (10 mL), dried over $MgSO_4$ and concentrated *in vacuo*, yielding a transparent liquid. This crude material was chromatographed on a silica gel column (Isolute SI column) using a gradient elution of 0 → 2% CH_3OH in $CHCl_3$. The title compound was obtained as a white solid (115 mg, 63%)

^1H NMR (300MHz, $CDCl_3$) δ 1.28-1.52 (4H, m, 3'-H and 4'-H), 1.69 (4H, m, 2'-H and 5'-H), 3.11 (2H, t, *J* = 6.4 Hz, 6'-H), 3.72 (2H, t, *J* = 7.3 Hz, 1'-H), 5.73 (1H, d, *J* = 7.8 Hz, 5-H), 7.12 (1H, d, *J* = 7.7 Hz, 6-H), 7.23-7.40 (9H, m,Ph-H), 7.50 (6H, m,Ph-H), 9.98 (1H, bs, 3-NH).

^{13}C NMR (75MHz, $CDCl_3$) δ 26.3 (3'-CH_2 or 4'-CH_2), 26.7 (3'-CH_2 or 4'-CH_2), 29.4 (2'-CH_2 or 5'-CH_2), 30.3 (2'-CH_2 or 5'-CH_2), 49.3 (1'-CH_2 or 6'-CH_2), 63.7 (1'-CH_2 or 6'-CH_2), 86.8 (1''-C), 102.5 (5-CH), 127.3 (Ph-CH), 128.2 (Ph-CH), 129.1 (Ph-CH), 144.8 (Ph-C), 144.9 (6-CH), 151.5 (2-C), 164.7 (4-C).

2D NMR spectra C-H and NOESY were recorded.

ES$^+$ m/z (%) 477 ([M+Na]$^+$, 100), 243 (Ph_3C^+, 58).

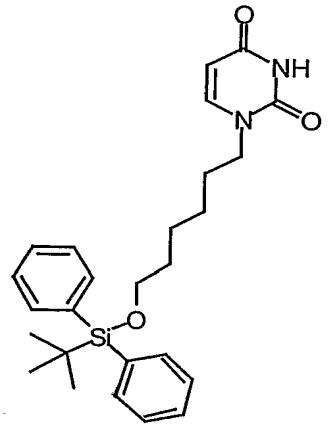
HRMS (ES$^+$*)* Found [M+NH_4]$^+$ 472.2592; $C_{29}H_{34}N_3O_3$ requires 472.2595.

IR (KBr) 3052, 2940, 1712 (weak), 198, 1666, 1468, 1426, 1359, 758, 704 cm^{-1}.

M.p. 155-156°C.

Example 6

1-(4'- *tert*-butyldiphenylsilyloxyhexyl)uracil



Uracil (0.091 g, 0.78 mmol) and caesium carbonate (0.169g, 0.52 mmol) were stirred in dry DMF (40 mL) at room temperature under nitrogen for 30 min. A mixture of 4-bromo- and 4-chloro-1-*O*-*tert*-butyldiphenylsilyl-hexanol(0.218 g, *ca* 0.56 mmol) in dry DMF (2 mL) was added drop-wise. The reaction mixture was heated at 50°C for 48h. The crude solution was partitioned between water (10 mL) and EtOAc (10 mL). The organic layer was further washed with brine (2 x 5 mL), then dried over $MgSO_4$ and concentrated *in vacuo* to give a transparent oil. This product was further purified by silica gel column chromatography (Isolute SI column) using a gradient elution of 0 → 5% CH_3OH in $CHCl_3$. The fractions with R_f = 0.03 (30% EtOAc/hexane) afforded the title compound as a transparent oil (0.103 g, *ca* 41%). A small amount of dialkylated product was isolated as a transparent film (15 mg, *ca* 3%) from the fractions with R_f = 0.28 (30% EtOAc/hexane).

^1H NMR (300MHz, $CDCl_3$) δ 1.11 (9H, s, *t*Bu-CH_3), 1.41 (4H, m, 3'-H and 4'-H), 1.67 (4H, m, 2'-H and 5'-H), 3.73 (4H, m, 1'-H and 6'-H), 5.76 (1H, dd, *J* = 2.0, 7.9 Hz, 5-H), 7.17 (1H, d, *J* = 7.9 Hz, 6-H), 7.46 (6H, m, Ph-CH), 7.73 (4H, m, Ph-CH), 9.80-9.92 (1H, bm, 3-NH).

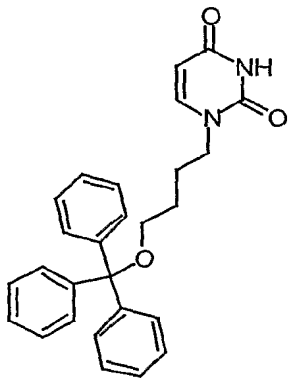
^{13}C NMR (75MHz, $CDCl_3$) δ 19.7 (*t*Bu-C), 25.9 (3'-CH_2 or 4'-CH_2), 26.6 (3'-CH_2 or 4'-CH_2), 27.3 (*t*Bu-CH_3), 29.5 (2'-CH_2 or 5'-CH_2), 32.8 (2'-CH_2 or 5'-CH_2), 49.3 (1'-CH_2), 64.1 (6'-CH_2), 102.5 (5-CH), 128.1 (Ph-CH), 130.0 (Ph-CH), 134.4 (Ph-C), 136.0 (Ph-CH), 144.9 (6-CH), 151.5 (2-C), 164.6 (4-C).

ES^+ m/z (%) 473 ($[M+Na]^+$, 100).

HRMS (ES^+) Found $[M+H]^+$ 451.2409; $C_{26}H_{35}N_2O_3Si$ requires 451.2411.

Example 7

1-(4'-Trityloxybutyl)uracil



1-(4'-Hydroxybutyl)uracil (0.083 g, 0.45 mmol), trityl chloride (0.140 g, 0.50 mmol) and DMAP (5 mg, 0.05 mmol) were stirred in dry pyridine (6 mL) at 50°C under nitrogen for 64 h. The reaction mixture was left to cool to room temperature and then partitioned between cold water (20 mL) and DCM (15 mL, 2 x 10 mL). The organic extracts were combined, washed with brine (20 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Further purification by silica gel column chromatography (Isolute SI column) using a gradient elution of 0 → 3% CH_3OH in $CHCl_3$. The fractions with R_f = 0.58 (10% $CH_3OH/CHCl_3$) yielded the title compound as a white solid (0.165 g, 86%).

^1H NMR (300MHz, $CDCl_3$) δ 1.27 (2H, m, 3'-H), 1.38 (2H, m, 2'-H), 3.23 (2H, t, *J* = 6.1 Hz, 4'-H), 3.81 (2H, t, *J* = 7.1 Hz, 1'-H), 5.78 (1H, dd, *J* = 2.2, 7.9 Hz, 5-H), 7.18 (1H, d, *J* = 7.9 Hz, 6-H), 7.17-7.56 (15, m, Ph-H), 9.25 (1H, bs, 3-NH).

^{13}C NMR (75MHz, $CDCl_3$) δ 26.5 (2'-CH_2), 29.2 (3'-CH_2), 49.2 (1'-CH_2), 63.0 (4'-CH_2), 87.0 (Ph_3C), 102.5 (C-5), 127.4 (Ph-CH), 128.3 (Ph-CH), 129.0 (Ph-CH), 144.5(Ph-C), 144.9 (6-C), 151.2 (2-C), 164.1 (4-C).

2D NMR spectra C-H and NOESY were recorded.

ES^+ m/z (%) 449 ([M+Na]+, 40), 55 (100).

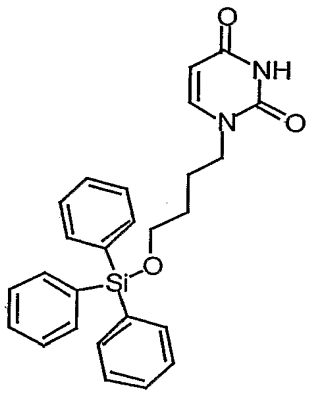
HRMS (ES^+) Found $[M+Na]^+$ 449.1838. $C_{20}H_{14}N_2NaO_3$ requires 449.1836.

IR (KBr) 3045, 1681 (C=O), 1666 (C=O), 1448, 1074, 760, 706 cm^{-1}.

M.p. 68-69°C.

Example 8

1-(4'-Triphenylsilyloxybutyl)uracil



1-(4'-Hydroxybutyl)uracil (0.048 g, 0.26 mmol) was dissolved in dry pyridine (1.5 mL) and cooled in an ice-salt bath. A solution of triphenylsilyl chloride (0.099 g, 0.34 mmol) in dry pyridine (1.5 mL) was added drop-wise. The reaction mixture was kept at 0°C under nitrogen for 2h30. CH_3OH (15 μL) was added and after 10 min the solution was concentrated on the rotary evaporator. The resultant crude transparent oil was purified by silica gel chromatography using a gradient elution of 0 → 3% CH_3OH in

$CHCl_3$. The title compound was obtained as a white solid (0.085 g, 74%) from the fractions with R_f = 0.50 (10% $CH_3OH/CHCl_3$).

^1H NMR (300MHz, $CDCl_3$) δ 1.65 (2H, m, 3'-H), 1.80 (2H, m, 2'-H), 3.73 (2H, t, *J* = 7.2 Hz, 1'-H), 3.89 (2H, t, *J* = 5.9 Hz, 4'-H), 5.66 (1H,dd, *J* = 1.9, 7.8 Hz, 5-H), 7.02 (1H, d, *J* = 7.8 Hz, 6-H), 7.44 (9H, m, Ph-H), 7.65 (6H, m, Ph-H), 9.59 (1H, bs, 3-NH).

^{13}C NMR (75MHz, $CDCl_3$) δ 26.1 (2'-CH_2), 29.4 (3'-CH_2), 49.0 (1'-CH_2), 63.6 (4'-CH_2), 102.5 (5-C), 128.4 (Ph-CH), 130.6 (Ph-CH), 134.4 (Ph-C), 135.8 (Ph-CH), 144.9 (6-C), 151.4 (2-C), 164.4 (4-C).

2D NMR spectra C-H and NOESY were recorded.

ES$^+$ m/z (%) 465 ($[M+Na]^+$, 40), 55(100).

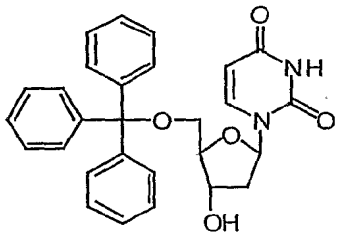
HRMS (ES$^+$) Found $[M+H]^+$ 443.1787; $C_{26}H_{26}N_2O_3Si$ requires 443.1785.

IR (KBr) 3053, 1714 (C=O), 1682 (C=O), 1427, 1117, 703 cm^{-1}.

M.p. 131-132°C.

Example 9

1-(4-hydroxy-5-[(trityloxy)methyl]tetrahydro-2-furanyl]-1,2,3,4-tetrahydro-2,4-pyrimidinedione or 5'-*O*-trityl-2'-deoxyuridine



2'-Deoxyuridine,(1.00g, 4.39mmol) and triphenylmethylchloride (1.34g, 4.83mmol) were stirred in dry pyridine (20ml) overnight at 50°C under an atmosphere of nitrogen. The reaction mixture was then poured into ice-H_2O (100ml) with vigorous stirring and filtered. The precipitate was dissolved in EtOAc (100ml) and the solution was washed with 0.5M HCl (100ml) and H_2O (100ml), dried (Na_2SO_4) and reduced *in vacuo*. The residue was washed with toluene to leave the title compound (1.99g, 97%)

as a pale yellow solid. For analytical purposes, the compound was purified by gradient flash column chromatography, eluting with 5→10% MeOH/$CHCl_3$.

^1H NMR (300MHz;$CDCl_3$): δ 2.34 (1H, m, 2'-H), 2.45 (1H, m, 2'-H), 3.51 (2H, ddd, *J* = 3.5, 8.6, 10.6 Hz, 5'-H), 4.12 (1H, dd, *J* = 3.6, 7.2 Hz, 4'-H), 4.64 (1H, m, 3'-H), 5.47 (1H, d, *J* = 8.1 Hz, 5-H), 6.40 (1H, t, *J* = 6.3 Hz, 1'H), 7.22-7.49 (15H, m, Ph-H), 7.86 (1H, d, *J* = 8.1Hz, 6-H), 9.37 (1H, s, 3-NH);

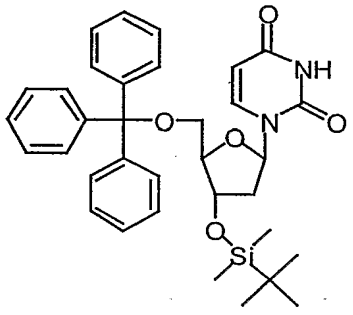
^{13}C NMR (75MHz;$CDCl_3$): δ 41.60 (2'-CH_2), 63.53 (5'-CH_2), 71.84 (3'-CH), 85.49 (4'-CH), 86.43 (1'-CH), 88.03 (Ph_3C) , 127.92 (Ph-CH), 128.68 (Ph-CH), 129.49 (Ph-CH), 140.69 (6-CH), 143.67 (Ph-CH), 153.24 (2-C), 163.93 (4-C).

MS (AP^+., *m/z*): 243 (Tr^+, 100%);

R_f (10%MeOH/$CHCl_3$): 0.49;

Example 10

1-[4-(*tert*-Butyl-dimethyl-silanyloxy)-5-trityloxymethyl-tetrahydro-furan-2-yl]-1*H* pyrimidine-2,4-dione or 3'-*O*-*tert*-Butylsilyl-5'-*O*-trityl-2'-deoxyuridine



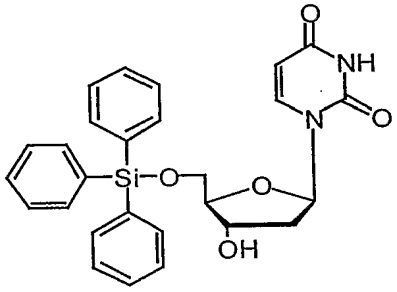
5'-*O*-trityl-2'deoxyuridine (0.70g, 1.49mmol) in dry DMF (3ml) was added drop-wise under an atmosphere of nitrogen, with ice bath cooling, to a stirred solution of *tert*-butyldimethylsilylchloride (0.25g, 1.65mmol) and imidazole (0.22g, 3.28mmol) in dry DMF (3ml). The mixture was allowed to warm to room temperature and stirred overnight. H_2O (10ml) was added

(10ml) and the mixture was extracted with Et_2O (2x50ml). The combined extracts were washed with saturated $NaHCO_3$ (50ml) and H_2O (50ml), dried (Na_2SO_4) and reduced *in vacuo*. A flash silica column eluting with 3% MeOH/$CHCl_3$ gave the title compound (0.65g, 74%) as white foam.

^1H NMR (300MHz;$CDCl_3$): δ -0.05 [3H, s, $Si(C\underline{H}_3)_2$] and 0.00 [3H, s, $Si(C\underline{H}_3)_2$], 0.85 [9H, s, $C(C\underline{H}_3)_3$], 2.12-2.20 (1H, m, 2'-H), 2.31-2.39 (1H, m, 2'-H), 3.33 (1H, dd, *J* = 2.8, 10.7 Hz, 5'-H), 3.46 (1H, dd, *J* = 2.9, 10.7Hz, 5'-H), 3.92 (1H, dt, *J* = 2.8, 4.4Hz, 4'-H), 4.51 (1H, dd, *J* = 4.9, 10.9Hz, 3'-H), 5.34 (1H, d, *J* = 8.1Hz, 5-H), 6.26 (1H, t, *J* = 6.0Hz, 1'-H), 7.23-7.39 (15H, m, Ph-H), 7.85 (1H, d, *J* = 8.1Hz, 6-H), 9.11 (1H, s, 3-NH) ;
^{13}C NMR (75MHz; $CDCl_3$): -4.49 and –4.20 ($Si(\underline{C}H_3)_2$), 18.37[$\underline{C}(CH_3)_3$], 22.06 and 26.17 [$C(\underline{C}H_3)_3$], 42.23 (2'-CH_2), 62.27 (5'-CH_2), 71.38(3'-CH), 85.55 (4'-CH) 86.83 (1'-CH), 87.89 (Ph_3C), 102.70 (5-CH), 127.91 (Ph-CH), 128.48 (Ph-CH), 129.15 (Ph-CH), 140.62 (6-CH), 143.59 (Ph-CH), 150.68 (2-C), 163.81 (4-C);
MS (AP^+., *m/z*): 243 (Tr^+, 50%), 341 (M-Tr^+, 75%), 607 (M+Na^+, 100%)
R_f (3%MeOH/$CHCl_3$): 0.33;

Example 11

5'-O-Triphenylsilyl-2'-deoxyuridine



A solution of triphenylsilyl chloride (0.437 g, 1.48 mmol) in dry pyridine (4 mL) was added drop-wise to a solution of 2'-deoxyuridine (0.278 g, 1.22 mmol) in dry pyridine (4 mL) previously cooled in an ice-salt bath. The reaction mixture was kept at 0°C for 1h. The reaction was monitored by TLC (10% CH_3OH in $CHCl_3$) and quenched with CH_3OH (50μL). The

solvent was removed under reduced pressure to give a crude yellow liquid which was further purified by silica gel column chromatography (Isolute SI column) using a gradient elution of 0 → 10% CH_3OH in $CHCl_3$. The fractions with Rf = 0.30 (10% $CH_3OH/CHCl_3$) were combined and concentrated to yield the title compound as a white crystalline solid (0.506 g, 85%).

^1H NMR (300MHz, $CDCl_3$) δ 2.25 (1H, m, 2'-H), 2.44 (1H, m, 2'-H), 2.95 (1H, bs, 3'-OH), 3.93-4.27 (3H, m, 5'-H and 4'-H), 4.60 (1H, m, 3'-H), 5.19 (1H, d, *J* = 8.2 Hz, 5-H), 6.41 (1H, t, *J* = 6.4 Hz, 1'-H), 7.35-7.73 (15H, m, Ph-H), 7.80 (1H, d, *J* = 8.1 Hz, 6-H), 9.46 (1H, bs, 3-NH).

^{13}C NMR (75MHz, $CDCl_3$) δ 41.6 (2'-CH_2), 63.8 (5'-CH_2), 71.7 (3'-CH), 85.3 (1'-CH), 87.3 (4'-CH), 102.7 (5-CH), 128.6 (Ph-CH), 131.1 (Ph-CH), 133.3 (Ph-C), 135.8 (Ph-CH), 140.5 (6-CH), 150.9 (2-C), 163.9 (4-C).

ES^+ m/z (%) 509 ($[M+Na]^+$, 100).

2D NMR spectra H-H and C-H were recorded.

ES^+ m/z (%) 509 ($[M+Na]^+$, 78), 151 (100).

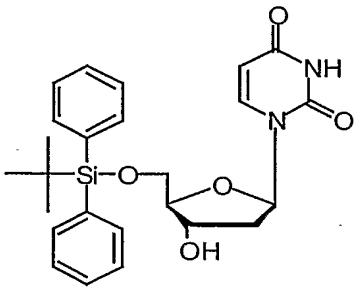
HRMS (ES^+) Found $[M+Na]^+$ 509.1504; $C_{27}H_{26}N_2O_5Si$ requires 509.1503.

Anal. (%) found C 65.01, H 5.27, N 5.62.

Calcd for $C_{27}H_{26}N_2O_5Si$, 0.32 HCl C 65.09, H 5.32, N 5.62.

Example 12

5'-*O*-*tert*-Butyldiphenylsilyl-2'-deoxyuridine



2'-Deoxyuridine (0.530 g, 2.32 mmol) was dissolved in dry DMF (5mL) under nitrogen and the solution was cooled in an ice-salt bath. A solution of *tert*-butyldiphenylsilyl chloride (0.710 g, 2.58 mmol) and imidazole

(0.342 g, 5.69 mmol) in dry DMF (4 mL) was then added drop-wise. The reaction mixture was stirred at 0°C for 2h and then at room temperature for 15h. The reaction was quenched by addition of water (15 mL). The crude mixture was extracted with $CHCl_3$ (2 x 15 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated *in vacuo* to give a transparent oil (0.419 g). This oil was chromatographed on a silica gel column (Isolute SI column) eluted with a gradient of 0 → 10% CH_3OH in $CHCl_3$. The fractions with R_f = 0.26 (10% $CH_3OH/CHCl_3$) were gathered and concentrated to afford the title compound as a white crystalline solid (0.823 g, 76%).

^1H NMR (300MHz, $CDCl_3$) δ 1.14 (9H, m, *t*Bu-H), 2.27 (1H, m, 2'-H), 2.50 (1H, m, 2'-H), 2.69 (1H, bs, 3'-OH), 3.90 (1H, m, 4'-H), 4.05 (2H, m, 5'-H), 4.60 (1H, m, 3'-H), 5.52 (1H, d, *J* = 8.1 Hz, 5-H), 6.41 (1H, t, *J* = 6.4 Hz, 1'-H), 7.48 (6H, m, Ph-H), 7.70 (4H, m, Ph-H), 7.87 (1H, d, *J* = 8.1 Hz, 6-H), 9.34 (1H, bs, 3-NH).

^{13}C NMR (75MHz, $CDCl_3$) δ 19.7 (*t*Bu-C), 27.4 (*t*Bu–CH_3), 41.7 (2'-CH_2), 64.1 (5'-CH_2), 71.9 (3'-CH), 85.4 (1'-CH), 87.5 (4'-CH), 102.6 (5-CH), 128.4 (Ph-CH), 128.5 (Ph-CH), 130.6 (Ph-CH), 132.7 (Ph-C), 133.1 (Ph-C), 135.8 (Ph-CH), 136.0 (Ph-CH), 140.5 (6-CH), 150.9 (2-C), 163.9 (4-C).

2D NMR spectra H-H and C-H were recorded.

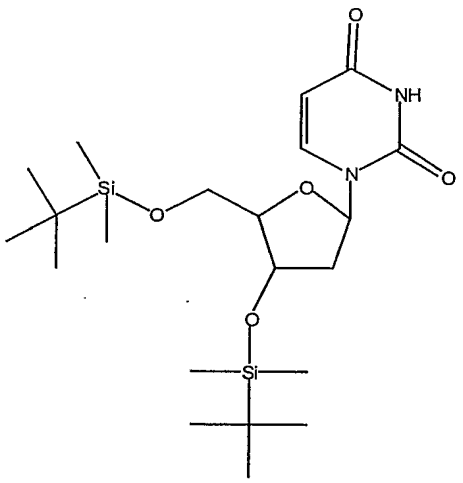
ES$^+$ m/z (%) 489 ([M+Na]$^+$, 100).

Anal. (%) found C 61.61, H 6.23, N 5.72.

Calcd for $C_{25}H_{30}N_2O_5Si$, 0.58 HCl C 61.56, H 6.32, N 5.74.

Example 13

3',5'-O-bis*tert*butyldimethylsilyl-2'-deoxyuridine

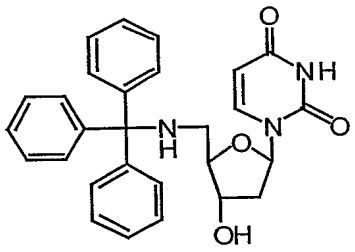


A solution of *t*-butyl dimethylsilylchloride (2.18 g, 14.46 mmol) and imidazole (1.07 g, 28.92 mmol) in DMF (30 ml) was added slowly (drop wise) to a stirred solution of 2'-deoxyuridine (3 g, 13.15 mmol) in dry DMF (40 ml), with ice-bath cooling at 0°C, under atmosphere of nitrogen.
After 2 hours, H_2O (100 ml) was added and the mixture was extracted with AcOEt (3×100 ml). The combined extracts were washed with saturated $NaHCO_3$ (2×100 ml), dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography and the title compound was isolated as a white amorphous solid from the fractions with Rf= 0.65 (10% CH_3OH in $CHCl_3$) .

^{1}H-NMR (300MHz, $CDCl_3$) δ 0.2 (s, 12H, tBu[C*H*$_3$]$_2$Si), 1.0 (s, 18H, *tBu*[CH_3]$_2$Si), 2.5 (1H, m, 2'-H), 2.2 (1H, m, 2'-H), 3.06 (1H, d, *J*=5.0 Hz, 3'-H), 3.95 (1H, dd, *J*=11.5 Hz, 2.2 Hz, 5'-H), 4.01 (1H, dd, *J*=11.5, 2.6 Hz, 5'-H), 4.15 (1H, m, 4'-H), 5.78 (1H, d, *J*=8.23 Hz, 5-H), 6.45 (1H, t, *J*=6.95 Hz, 1'-H), 8.02 (1H, d, *J*=8.2 Hz, 6-H), 9.5 (1H, s, 3-NH),.
^{13}C-NMR (75MHz, $CDCl_3$) δ 163.9 (4-C), 150.9 (2-C), 140.8 (6-CH), 102.7 (5-CH), 87.9 (4'-CH), 85.8 (1'-CH), 72.5 (3'-CH), 63.7 (5'-CH_2), 42.0 (2'-CH_2), 26.3 (CH_3), 18.8 (CH_3).

Example 14

5'-Tritylamino-2',5'-dideoxyuridine



5'-Amino-2',5'-dideoxyuridine (0.200 g, 0.88 mmol) was taken in dry pyridine (5 mL) and the mixture was sonicated for a few minutes. Trityl chloride (0.278 g, 1.00 mmol) was added and the reaction mixture was stirred at 50°C overnight. The reaction was then quenched with water (20 mL). The crude mixture was extracted with DCM (3 x 10 mL). The organic layers were combined, washed with water (10 mL), dried over $MgSO_4$ and concentrated on the rotary evaporator. The resultant brown oil was further purified by silica gel column chromatography (Isolute SI column) using a gradient elution of 0 → 10% CH_3OH in $CHCl_3$. The fractions with R_f = 0.28 (10% $CH_3OH/CHCl_3$) were pooled and evaporated to dryness to yield the title compound as a white solid (0.202 g, 49%).

^1H NMR (300MHz, $CDCl_3$) δ 2.07 (2H, m, 2'-H), 2.28-2.53 (2H, m, 5'-H), 2.73 (1H, dd, *J* = 3.5, 12.1 Hz, 1''-NH), 2.97 (1H, bs, 3'-OH), 4.19 (1H, m, 4'-H), 4.33 (1H, m, 3'-H), 5.72 (1H, d, *J* = 8.1 Hz, 5-H), 6.36 (1H, t, *J* = 6.4 Hz, 1'-H), 7.14 (1H, d, *J* = 8.1 Hz, 6-H), 7.23-7.43 (9H, m, Ph-H), 7.57 (6H, m, Ph-CH), 9.47 (1H, bs, 3-NH).

^{13}C NMR (75MHz, $CDCl_3$) δ 40.8 (2'-CH_2), 46.6 (5'-CH_2), 71.1 (2''-C), 73.0 (3'-CH), 85.4 (1'-CH), 86.7 (4'-CH), 103.2 (5-CH), 127.0 (Ph-CH), 128.4 (Ph-CH), 129.0 (Ph-CH), 139.8 (6-CH), 145.8 (Ph-C), 150.7 (2-C), 163.7 (4-C).

2D NMR spectra H-H and C-H were recorded.

ES^+ m/z (%) 243 (Ph_3C^+, 100), 470 ($[M+H]^+$, 4), 492 ($[M+Na]^+$, 23).

HRMS (ES^+) Found $[M+H]^+$ 470.2076; $C_{28}H_{28}N_3O_4$ requires 470.2074.

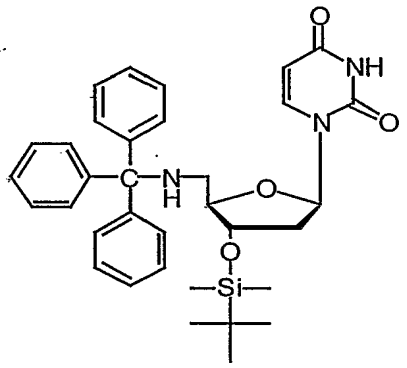
M.p. 132-134°C.

Anal (%) found C 68.79, H 5.55, N 8.59;

Calcd for $C_{28}H_{27}N_3O_4$, 0.53 HCl C 68.79, H 5.68, N 8.60.

Example 15

3'-*O*-*tert*butyldimethylsilyl-5'-Tritylamino-2',5'-dideoxyuridine



A solution of 5'-tritylamino-2',5'-dideoxyuridine (0.172 g, 0.37 mmol) in anhydrous DMF (2 mL) was added drop-wise to an ice cold solution of *tert*-butyl dimethylsilyl chloride (68 mg, 0.45 mmol) and imidazole (60 mg, 0.88 mmol) in anhydrous DMF (2 mL). The reaction mixture was stirred at 0°C for 2h and at room temperature for a further 20h. It was then partitioned between water (10 mL) and Et_2O (2 x 20 mL). The combined organic layers were washed with a saturated aqueous solution of $NaHCO_3$ (15 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The resultant white solid was further purified by column chromatography (Isolute SI column) using a gradient elution of 0 → 10% CH_3OH in $CHCl_3$. The fractions with R_f = 0.69 (10% $CH_3OH/CHCl_3$) were pooled and evaporated to dryness to yield the title compound as a white solid (154 mg, 72%).

^1H NMR (300MHz, $CDCl_3$) δ 0.00-0.02 (6H, 2 x s, $Si(CH_3)_2$), 0.86 (9H, s, $C(CH_3)_3$), 1.90 (2H, m, 2'-H), 2.11-2.35 (2H, m, 5'-H), 2.59 (1H, bd, $J \approx 13$ Hz, 1''-NH), 4.06 (2H, m, 3'-H and 4'-H), 5.65 (1H, d, J = 8.1 Hz, 5-H), 6.25 (1H, t, J = 6.3 Hz, 1'-H), 7.07 (1H, d, J = 8.1 Hz, 6-H), 7.14-7.37 (9H, m,Ph-H), 7.48 (6H, m,).

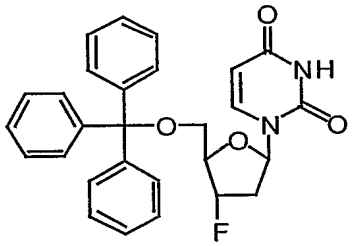
^{13}C NMR (75MHz, $CDCl_3$) δ -4.4 ($SiCH_3$), -4.2 ($SiCH_3$), 18.4 ($C(CH_3)_3$), 26.1 ($C(CH_3)_3$), 41.4 (2'-CH_2), 46.5 (5'-CH_2), 71.2 (2''-C), 73.3 (3'-CH), 85.6 (1'-CH), 87.3 (4'-CH), 103.1 (5-CH), 127.0 (Ph-CH), 128.4 (Ph-CH), 129.0 (Ph-CH), 139.7 (6-CH), 145.9 (Ph-C), 150.6 (2-C), 163.7 (4-C).

ES$^+$ m/z (%) 584 ([M+H]$^+$), 606 ([M+Na]$^+$).

HRMS (ES$^+$*)* Found [M+H]$^+$ 584.2938; $C_{34}H_{42}N_3O_4Si$ requires 584.2939.

Example 16

1-(4-Fluoro-5-trityloxymethyl-tetrahydro-furan-2-yl)2,3-dihydro-1*H*-pyrimidin-4-one or 3'-Fluoro-5'-*O*-trityl-2',3'-dideoxyuridine



3'-Fluoro-2',3'-dideoxyuridine (0.3g, 1.30mmol) and triphenylmethyl chloride (0.44g, 1.57mmol) were stirred in dry pyridine (20ml) overnight at 50°C under an atmosphere of nitrogen. The reaction mixture was then poured into ice-H_2O (50ml) with vigorous stirring and filtered. The precipitate was dissolved in EtOAc (50ml) and the solution was washed with 0.5M HCl (50ml) and H_2O (50ml) dried (Na_2SO_4) and reduced *in vacuo* to obtain a crude product, which was purified by gradient flash column chromatography eluting with 2→6% MeOH/$CHCl_3$ to obtain the title compound as a white solid (0.48g, 77%).

^{1}H NMR(300MHz;$CDCl_3$): δ 2.27-2.50 (1H, m, 2'-H), 2.78-2.92 (1H, m, 2'-H), 3.53-3.63 (2H, m, 5'H), 4.41-4.51 (1H, d, *J* = 27.3 Hz, 4'-H), 5.33-5.53 (2H, m,3',5-H), 6.50-6.55 (1H, m, 1'-H), 7.46 (15H, m, Ph-H), 7.80 (1H, d, J 8.1, 6-H);

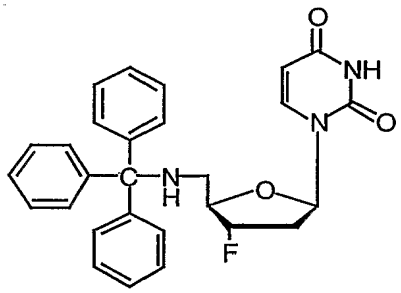
^{13}C NMR(75MHz;$CDCl_3$): δ 39.43 and 39.71 (2'-CH_2), 63.75 and 63.89 (5'-CH_2), 84.54 and 84.88 (4'-CH), 85.38 (1'-CH), 88.27 (Ph-C) -, 93.44 (Ph-CH), 95.80 (Ph-CH), J 178.48, 3'-CH), 103.08 (5-CH), 128.06 (Ph-CH), 128.58 (Ph-CH), 129.00 (Ph-CH), 140.18 (6-CH), 143.31 (Ph-C), 150.67 (2-C), 163.53 (4-C);

^{19}F NMR(282MHz;$CDCl_3$):δ -174.26;

MS (CI/NH_3., *m/z*): 473.2 ($M+H^+$, 50%), 490.3 ($M+NH_4^+$, 80%);
HRMS (EI., M^+): Calculated for $C_{28}H_{25}N_2O_4F$, requires 472.1798, found 472.1797.
IR_{vmax}/cm^{-1} (KBr): 703 (s) and 763(s) (Aromatic, monosubstituted), 1107.9 (C-F), 1689.3 (C=O) and 1702.3 (C=O).R_f (10% MeOH / $CHCl_3$): 0.52.
Mp: 128-130°C.

Example 17

3'-Fluoro-5'-tritylamino-2',3',5'-trideoxyuridine

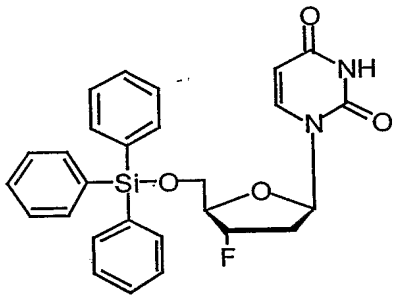


The title compound was obtained as a light yellow crystalline solid (91 mg, 32%) from the reaction of the corresponding amine (0.137 g, 0.59 mmol) and trityl chloride (0.199 g, 0.66 mmol) in dry pyridine (4mL). The procedure was similar to that followed for the preparation of the 3' hydroxy analogue 5'tritylamino-2',5'-dideoxyuridine (WSP871, see example 14).

^1H NMR (300MHz, $CDCl_3$) δ 1.87-2.13 (2H, m, 2'-H), 2.28 (1H, dd, *J* = 8.1, 12.0 Hz, 1''-NH), 2.57-2.78 (2H, m, 5'-H), 4.48 (1H, dm, *J* ≈ 25 Hz, 4'-H), 5.11 (1H, dd, *J* = 5.3, 53.7 Hz, 3'-H), 5.71 (1H, d, *J* = 8.1 Hz, 5-H), 6.37 (1H, dd, *J* = 5.6, 8.7 Hz, 1'-H), 6.98 (1H, d, *J* = 8.1 Hz, 6-H), 7.23-7.43 (9H, m, Ph-H), 7.53 (6H, m, Ph-CH), 9.39.(1H, s, 3-NH).
^{13}C NMR (75MHz, $CDCl_3$) δ 38.5 (d, *J* = 21.8 Hz, 2'-CH_2), 46.1 (d, *J* = 9.2 Hz, 5'-CH_2), 71.1 (Ph_3C), 85.2 (d, *J* = 25.3 Hz, 4'-CH), 85.5 (1'-CH), 94.4 (d, *J* = 179.9 Hz, 3'-CH), 103.6 (5-CH), 127.1 (Ph-CH), 128.5 (Ph-CH), 128.9 (Ph-CH), 139.3 (6-CH), 145.7 (Ph-C), 150.5 (2-C), 163.4 (4-C).
^{19}F NMR (282MHz, $CDCl_3$) δ -175.7 (m, 3'-F).
ES$^+$ m/z (%) 243 (Ph_3C^+, 93), 494 ($[M+Na]^+$, 92).

Example 18

3'-Fluoro-5'-O-triphenylsilyl-2',3'-dideoxyuridine



The title compound was synthesised following a similar procedure as described for Example 11. 3'-Fluoro-2',3'-dideoxyuridine (0.214 g, 0.93 mmol) was reacted with triphenylsilyl chloride (0.332 g, 1.12 mmol) in dry pyridine (7 mL) for 3h. to yield the title compound as a white solid (0.274 g, 60%).

^{1}H NMR (300MHz, $CDCl_3$) δ 2.19 (1H, m, 2'-H), 2.67 (1H, m, 2'-H), 4.11 (2H, m, 5'-OH), 4.36 (1H, d, *J* = 27.1 Hz, 3'-H), 5.22 (1.5H, m, 4'-H and 5-H), 5.40 (0.5H, d, *J* = 4.8 Hz, 4'-H), 6.50 (1H, dd, *J* = 5.4, 9.1 Hz, 1'-H), 7.41-7.75 (16H, m, 6-H and Ph-H), 9.04 (1H, bs, 3-NH).

^{13}C NMR (75MHz, $CDCl_3$) δ 39.5 (d, *J* = 20.7 Hz, 2'-CH_2), 64.3 (d, *J* = 11.5 Hz, 5'-CH_2), 85.2 (1'-CH), 85.4 (d, *J* = 24.7 Hz, 4'-CH), 94.9 (d, *J* = 178.7 Hz, 3'-CH), 103.1 (5-CH), 128.8 (Ph-CH), 131.2 (Ph-CH), 133.0 (Ph-C), 135.7 (Ph-CH), 140.1 (6-CH), 150.7 (2-C), 163.5 (4-C).

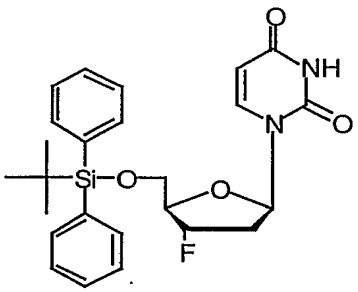
^{19}F NMR (282MHz, $CDCl_3$) δ -175.1 (m, 3'-F).

ES$^+$ m/z (%) 511 ([M+Na]$^+$, 5), 87 (100).

ES$^-$ m/z (%) 487 ([M-H$^+$], 31), 75 (100).

Example 19

3'-Fluoro-5'-*O-tert*-Butyldiphenylsilyl-2',3'-dideoxyuridine



The title compound was synthesised following a similar procedure as described for Example 12. 3'-Fluoro-2',3'-dideoxyuridine (0.176 g, 0.77 mmol) was reacted with *tert*-butyldiphenylsilyl chloride (0.238 g, 0.87 mmol) and imidazole (0.116 g, 1.70 mmol) in dry DMF (4 mL) for 3h. Compound WSP948 was obtained as a white solid (0.331 g, 92%).

^{1}H NMR (300MHz, $CDCl_3$) δ 1.17 (9H, m, *t*Bu-H), 2.24 (1H, m, 2'-H), 2.78 (1H, m, 2'-H), 4.00 (2H, m, 5'-H), 4.38 (1H, d, *J* = 26.7 Hz, 4'-H), 5.34 (1H, dd, *J* = 4.9, 53.8 Hz, 3'-H), 5.56 (1H, d, *J* = 8.1 Hz, 5-H), 6.51 (1H, m, 1'-H), 7.43-7.60 (6H, m, Ph-H), 7.65-7.74 (4H, m, Ph-H), 7.27 (1H, d, *J* = 8.1 Hz, 6-H), 9.11 (1H, bs, 3-NH).

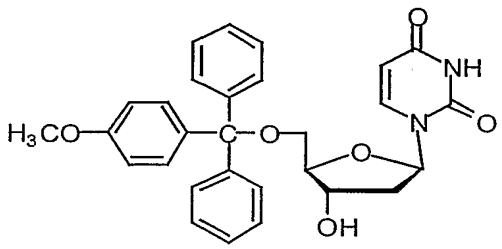
^{13}C NMR (75MHz, $CDCl_3$) δ 19.7 (*t*Bu-C), 27.4 (*t*Bu–CH_3), 39.7 (d, *J* = 21.3 Hz, 2'-CH_2), 64.1 (d, *J* = 10.9 Hz, 5'-CH_2), 85.4 (1'-CH), 85.6 (d, *J* = 24.7 Hz, 4'-CH), 94.7 (d, *J* = 178.7 Hz, 3'-CH), 103.2 (5-CH), 128.5 (Ph-CH), 128.6 (Ph-CH), 130.7 (Ph-CH), 132.2 (Ph-C), 132.8 (Ph-C), 135.7 (Ph-CH), 136.0 (Ph-CH), 140.0 (6-CH), 150.6 (2-C), 163.5 (4-C).

^{19}F NMR (282MHz, $CDCl_3$) δ -175.5 (m, 3'-F).

ES$^-$ m/z (%)467 ([M-H$^+$], 53), 75 (100).

Example 20

5'-*O*-*para*methoxytrityl-2'-deoxyuridine



4-Methoxytrityl (0.610g, 1.98 mmol) was added to a solution of 2'-deoxyuridine (0.410g, 1.80 mmol) in anhydrous pyridine (10 mL). The reaction mixture was stirred at 50°C for 40h. The crude mixture was partitioned between water (40 mL) and DCM (2 x 40 mL). The organic layers were combined, washed with water (2 x 80 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The resultant yellow oil was further purified by silica gel column chromatography (using Jones Chromatography Isolute SI columns). The column was eluted with a gradient of 0 → 5% CH_3OH in $CHCl_3$. The fractions with R_f = 0.28 (10% $CH_3OH/CHCl_3$) yielded the title compound as a white crystalline solid (0.625 g, 69%).

^1H NMR (300MHz, $CDCl_3$) δ 2.27 (1H, m, 5'-H), 2.42 (1H, m, 5'-H), 2.57 (1H, bs, 3'-OH), 3.42 (2H, m, 2'-H), 3.76 (3H, s, OCH_3), 4.00 (1H, m, 4'-H), 4.54 (1H, m, 3'-H), 5.37 (1H, d, *J* = 8.1 Hz, 5-H), 6.29 (1H, t, *J* = 6.3 Hz, 1'-H), 6.82 (2H, m, Ar-H), 7.18-7.38 (12H, m, Ar-H), 7.74 (1H, d, *J* = 8.1 Hz, 6-H), 9.20 (1H, bs, 3-NH).

^{13}C NMR (75MHz, $CDCl_3$) δ 41.6 (2'-CH_2), 55.7 (OCH_3), 63.5 (5'-CH_2), 71.9 (3'-CH), 85.5 (4'-CH), 86.5 (1'-CH), 87.8 (Ar_3C), 102.7 (5-CH), 113.8 (Ar-CH), 127.7 (Ar-CH), 128.5 (Ar-CH), 128.8 (Ar-CH), 130.8 (Ar-CH), 135.1 (Ar-C), 140.6 (6-CH), 144.1 (Ar-C), 144.3 (Ar-C), 150.8 (2-C), 159.3 (Ar-C), 163.7 (4-C).

ES^+ m/z (%) 523 ($[M+Na]^+$, 100)

HRMS (ES^+) Found $[M+Na]^+$ 523.1848; $C_{29}H_{28}N_2O_6Na$ requires 523.1845.

IR (KBr) 3208, 3054, 1714, 1694, 1682, 1507, 1470, 1250, 1092, 1035, 759 cm^{-1}.

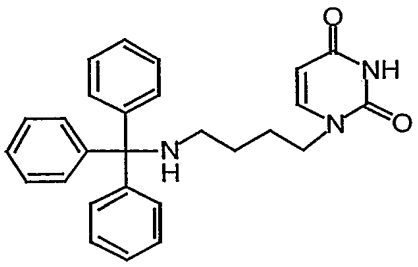
M.p. 96-97°C.

Anal (%) found C 62.17, H 5.05, N 4.85, Cl 8.86;

Calcd for $C_{29}H_{28}N_2O_6$, 1.43 HCl, 0.40 H_2O C 62.21, H 5.44, N 5.00, Cl 9.06.

Example 21

1-(4'-Tritylaminobutyl)uracil



Trityl chloride (0.358 g, 1.28 mmol) was added to a solution of 1-(4'-aminobutyl)uracil (contaminated by 11% 1,3-bis (4'-aminobutyl)uracil) (0.168 g) in anhydrous pyridine (15 mL). The reaction mixture was heated at 50°C for 44h, left to cool to room temperature, poured into cold water (50 mL) and extracted with DCM (3 x 25 mL). The organic extracts were washed with brine (40 mL), dried over Na_2SO_4 and concentrated *in vacuo*. Further purification was carried out by silica gel column chromatography, using Jones Chromatography Isolute SI columns with a gradient elution of 0 → 5% CH_3OH in $CHCl_3$. The fractions with R_f = 0.51 (10% $CH_3OH/CHCl_3$) gave the title compound as a white crystalline solid (94 mg, *ca* 24%).

^1H NMR (300MHz, $CDCl_3$) δ 1.58 (3H, m, 3',5'-H), 1.80 (2H, m, 2'-H), 2.23 (2H, t, *J* = 6.7 Hz 4'-H), 3.75 (2H, t, *J* = 7.2 Hz, 1'-H), 5.73 (1H, d, *J* = 7.9 Hz, 5-H), 7.13 (1H, d, *J* = 7.9 Hz, 6-H), 7.19-7.40 (9H, m, Ph-H), 7.53 (6H, m, Ph-H), 8.53 (1H, bs, 3-NH).

^{13}C NMR (75MHz, $CDCl_3$) δ 27.3 (2'-CH_2), 28.1 (3'-CH_2), 43.5 (4'- CH_2), 49.3 (1'-CH_2), 71.3 (6'-C), 102.5 (5-CH), 126.7 (Ph-CH), 128.3 (Ph-CH), 129.0 (Ph-CH), 144.8 (6-CH), 145.5 (Ph-C), 151.0 (2-C), 163.7 (4-C).

2D NMR spectra H-H, C-H and NOESY were recorded.

ES^+ m/z (%) 426 ($[M+H]^+$, 18), 243 (PhC^+, 100).

HRMS (ES^+) Found $[M+H]^+$ 426.2176; $C_{27}H_{28}N_3O_2^+$ requires 426.2176.

IR (KBr) 3054, 1694, 1672, 1454, 768, 706 cm^{-1}.

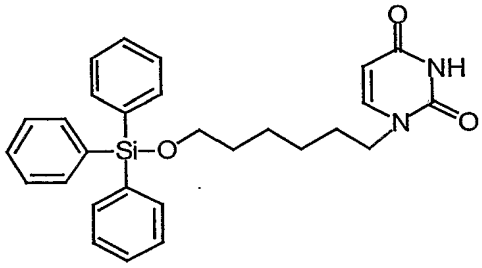
M.p. 219-220°C (dec.).

Anal (%) found C 73.10, H 6.12, N 9.45, Cl 3.61;

Calcd for $C_{27}H_{27}N_3O_2$, 0.48 HCl C 73.20, H 6.25, N 9.48, Cl 3.84.

Example 22

1-(6'-Triphenylsilyloxyhexyl)uracil



A solution of triphenylsilyl chloride (0.337 g, 1.14 mmol) in dry pyridine (3 mL) was added drop-wise to a solution of 1-(6'-hydroxyhexyl)uracil (0.200 g, 0.94 mmol) in dry pyridine (4 mL) cooled in an ice-salt bath. The reaction mixture was kept at 0°C under nitrogen for 3h30. As TLC monitoring evidenced the presence of unreacted starting material, triphenylsilyl chloride (0.325 g, 1.10 mmol) in dry pyridine (1 mL) was added. After a further 45 min at 0°C, the reaction had reached completion and was quenched with CH_3OH (0.1 mL). Removal of the solvent *in vacuo* afforded a crude yellow oil which was purified by silica gel chromatography using a Jones Chromatography Isolute SI column with a gradient elution of $0 \rightarrow 5\%$ CH_3OH in $CHCl_3$. The title compound was obtained as a white solid (0.140 g, 32%) from the fractions with R_f = 0.52 (10% $CH_3OH/CHCl_3$). Some starting material (0.130 g, 65%) was also recovered, possibly resulting from hydrolysis during the work up.

^1H NMR (300MHz, $CDCl_3$) δ 1.29-1.53 (4H, m, 3',4'-H), 1.67 (4H, m, 2',5'-H), 3.72 (2H, t, *J* = 7.3 Hz, 1'-H), 3.88 (2H, t, *J* = 6.3 Hz, 6'-H), 5.74 (1H, d, *J* = 7.8 Hz, 5-H), 7.12 (1H, d, *J* = 7.8 Hz, 6-H), 7.47 (9H, m, Ph-H), 7.70 (6H, m,Ph-H), 9.83 (1H, bs, 3-NH).

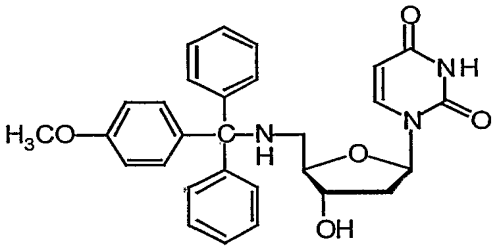
^{13}C NMR (75MHz, $CDCl_3$) δ 25.8 (3'-CH_2 or 4'-CH_2), 26.5 (4'-CH_2 or 3'-CH_2), 29.4 (2'-CH_2), 32.7 (5'-CH_2), 49.2 (1'-CH_2), 64.1 (6'-CH_2), 102.5 (5-CH), 128.3 (Ph-CH), 130.5 (Ph-CH), 134.8 (Ph-C), 135.8 (Ph-CH), 144.9 (6-CH), 151.4 (2-C), 164.5 (4-C).

ES$^+$ m/z (%) 493 ([M+Na]$^+$, 13).

HRMS (ES$^+$) Found $[M+H]^+$ 471.2100; $C_{28}H_{31}N_2O_3Si^+$ requires 471.2098.
IR (KBr) 3050, 2938, 2870, 1698, 1666, 1428, 1117, 700, 503 cm^{-1}.
M.p. 140-141°C.
Anal (%) found C 70.48, H 6.42, N 5.88, Cl 1.26;
Calcd for $C_{28}H_{30}N_2O_3Si$, 0.18 HCl C 70.47, H 6.37, N 5.87, Cl 1.34.

Example 23

5'-*para*Methoxytritylamino-2',5'-dideoxyuridine



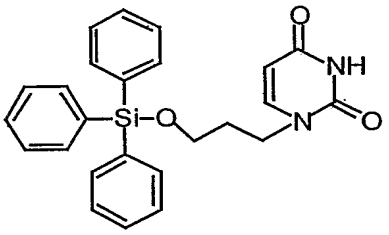
The procedure was similar to that described for example 20. 5'-Amino-2',5'-dideoxyuridine (0.204 g, 0.90 mmol) was reacted with 4-methoxytrityl (0.292 g, 0.99 mmol) to yield the title compound as a white solid (0.115g, 26%).

^{1}H NMR (300MHz, $CDCl_3$) δ 2.04 (2H, m, 2'-H), 2.29-2.48 (2H, m, 5'-H), 2.69 (1H, dd, *J* = 3.7, 12.1 Hz, 5'-N*H*), 3.83 (3H, s, O*CH*$_3$), 4.15 (1H, m, 4'-H), 4.30 (1H, m, 3'-H), 5.69 (1H, d, *J* = 8.1 Hz, 5-H), 6.32 (1H, t, *J* = 6.4 Hz, 1'-H), 6.88 (2H, m, Ar-H), 7.17 (1H, d, *J* = 8.1 Hz, 6-H), 7.21-7.61 (12H, m, Ar-H).
^{13}C NMR (75MHz, $CDCl_3$) δ 40.8 (2'-CH_2), 46.6 (5'-CH_2), 55.7 (OCH_3), 70.7 (Ar_3C), 73.0 (3'-CH), 85.4 (4'-CH), 86.8 (1'-CH), 103.2 (5-CH), 113.7 (Ar-CH), 126.9 (Ar-CH), 128.4 (Ar-CH), 128.9 (Ar-CH), 130.2 (Ar-CH), 138.0 (Ar-C), 139.9 (6-CH), 146.2 (Ar-C), 150.8 (2-C), 158.4 (Ar-C), 163.8 (4-C).
ES$^+$ m/z (%) 522 ($[M+Na]^+$, 27).
HRMS (ES$^+$) Found $[M+H]^+$ 500.2174; $C_{29}H_{30}N_3O_5^+$ requires 500.2180.
IR (KBr) 3052, 1713, 1694, 1682, 1666, 1650, 1506, 1250, 1034, 760 cm^{-1}.
M.p. 140-142°C.
TLC (10% $CH_3OH/CHCl_3$) R_f = 0.29.

Anal (%) found C 65.69, H 5.52, N 7.86, Cl 6.05;
Calcd for $C_{29}H_{29}N_3O_5$, 0.87 HCl C 65.56, H 5.67, N 7.91, Cl 5.81.

Example 24

1-(3'-Triphenylsilyloxypropyl)uracil



1-(3'-hydroxypropyl)uracil (0.193 g, 1.134 mmol) was dissolved in dry pyridine (4 mL) and cooled in an ice-salt bath. A solution of triphenylsilyl chloride (0.432 g, 1.46 mmol) in dry pyridine (3 mL) was added drop-wise. The reaction mixture was kept at 0°C under nitrogen for 4h30. As TLC monitoring evidenced the presence of unreacted starting material, more triphenyl slyl chloride (0.204 g, 0.69 mmol) in dry pyridine (1 mL) was added. After a further 15 min at 0°C, the reaction had reached completion and was quenched with CH_3OH (50 □L). Removal of the solvent *in vacuo* afforded a crude yellow oil which was purified by silica gel chromatography, using a Jones Chromatography Isolute SI column eluted with a gradient of 0 → 5% CH_3OH in $CHCl_3$. The title compound was obtained as a white solid (0.392 g, 81%) from the fractions with R_f = 0.52 (10% $CH_3OH/CHCl_3$). Some compound starting material was also recovered (34 mg, 18%).

^1H NMR (300MHz, $CDCl_3$) δ 2.01 (2H, m, 2'-H), 3.94 (4H, m, 1',3'-H), 5.54 (1H, d, *J* = 7.9 Hz, 5-H), 7.04 (1H, d, *J* = 7.9 Hz, 6-H), 7.51 (9H, m, Ph-H), 7.68 (6H, m, Ph-H), 9.49 (1H, bs, 3-NH).

^{13}C NMR (75MHz, $CDCl_3$) δ 31.5 (2'-CH_2), 32.7 (5'-CH_2), 46.4 (1'-CH_2), 60.3 (6'-CH_2), 102.1 (5-CH), 128.3 (Ph-CH), 130.8 (Ph-CH), 134.0 (Ph-C), 135.8 (Ph-CH), 145.6 (6-CH), 151.3 (2-C), 164.3 (4-C).

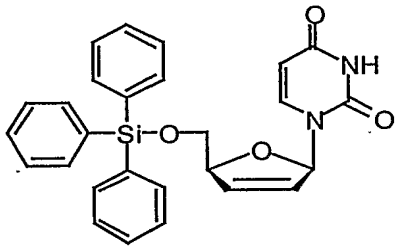
ES$^+$ m/z (%) 879 ($[2M+Na]^+$, 8), 451 ($[M+Na]^+$, 38), 87 (100).

HRMS (ES$^+$) Found $[M+NH_4]^+$ 446.1844; $C_{27}H_{28}N_3O_4Si^+$ requires 446.1844.

M.p. 150-151°C.

Example 25

5'-Triphenylsilyloxy-2',3'-dideoxydidehyrouridine



To a solution of 2',3'-dideoxydidehydrouridine (0.316 g, 1.50 mmol) in dry pyridine (5 mL) cooled in an ice-salt bath was added drop-wise a solution of triphenylsilyl chloride (0.595 g, 2.02 mmol) in dry pyridine (3 mL). The reaction mixture was kept at 0°C under nitrogen for 2h30. As TLC monitoring evidenced the presence of unreacted starting material, additional triphenylsilyl chloride (0.296 g, 1.00 mmol) in dry pyridine (1 mL) was added. After 1h30 min the reaction was quenched with CH_3OH (50 □L). Removal of the solvent *in vacuo* afforded a crude white gum which was purified by silica gel chromatography (Jones Chromatography Isolute SI column) eluted with 0 → 5% CH_3OH in $CHCl_3$. The title was obtained as a white solid (0.476 g, 68%) from the fractions with R_f = 0.57 (10% $CH_3OH/CHCl_3$).

^1H NMR (300 MHz, $CDCl_3$) δ 4.04 (1H, dd, *J* = 2.2, 11.7 Hz, 5'-H), 4.19 (1H, dd, *J* = 2.5, 11.7 Hz, 5'-H), 4.78 (1H, dd, *J* = 1.9, 8.1 Hz, 5-H), 4.98 (1H, m, 4'-H), 5.90 (1H, d, *J* = 5.7 Hz, 1'-H), 6.33 (1H, dd, *J* = 1.4, 4.5 Hz, 2'-H), 7.12 (1H, m, 3'-H), 7.40-7.65 (15H, m, Ph-H), 7.80 (1H, d, *J* = 8.1 Hz, 6-H), 8.99 (1H, bs, 3-NH).

^{13}C NMR (75 MHz, $CDCl_3$) δ 64.7 (5'-CH_2), 87.4 (1'-CH), 90.0 (4'-CH), 102.6 (5-CH), 127.1 (2'-CH), 128.6 (Ph-CH), 131.0 (Ph-CH), 133.3 (Ph-C), 134.9 (3'-CH), 135.8 (Ph-CH), 141.5 (6-CH), 151.2 (2-C), 163.6 (4-C).

ES$^+$ m/z (%) 491 ([M+Na]$^+$, 36), 119 (100).

HRMS (ES$^+$*)* Found [M+NH_4]$^+$ 446.1887; $C_{25}H_{28}N_3OSi^+$ requires 446.1894.

M.p. 73-74°C.

Anal (%) found C 67.75, H 5.04, N 5.84, Cl 1.89;

Calcd for $C_{27}H_{27}N_2O_4Si$, 0.25 HCl C 67.89, H 5.12, N 5.86, Cl 1.86.

Biological Examples

Example B1

Malaria whole cell assays

Parasite cultures

Two strains of *P.falciparum* are used in this study: The drug sensitive NF54 (an airport strain of unknown origin) and K1 (Thailand, chloroquine and pyrimethamine resistant). The strains are maintained in RPMI-1640 medium with 0.36 mM hypoxanthine supplemented with 25 mM HEPES, 25 mM $NaHC0_3$, neomycin (100 U/ml) and Albumax^R (lipid-rich bovine serum albumin) (GIBCO, Grand Island, NY) (5g/l), together with 5% washed human A+ erythrocytes. All cultures and assays are conducted at 37°C under an atmosphere of 4% $C0_2$, 3% 0_2 and 93% N_2. Cultures are kept in incubation chambers filled with the gas mixture. Subcultures are diluted to a parasitaemia of 0.1-0.5% and the medium changed daily.

Drug sensitivity assays

Antimalarial activity is assessed using an adaptation of the procedures described by Desjardins et al.(Antimicrob. Agents Chemother. 16(6):710-8, 1979), and Matile and Pink (In: Lefkovits, I. and Pernis, B. (Eds.). Immunological Methods. Academic Press, San Diego, pp. 221-234, 1990).

Stock drug solutions are prepared in 100% DMSO (dimethylsulfoxide) at 10 mg/ml, unless otherwise suggested by the supplier, and heated or sonicated if necessary. After use the stocks are kept at –20°C. The

compound is further diluted to the appropriate concentration using complete medium without hypoxanthine.

Assays are performed in sterile 96-well microtiter plates, each well containing 200 µl of parasite culture (0.15% parasitemia, 2.5% hematocrit) with or without serial drug solutions. Seven 2-fold dilutions are used covering a range from 5 µg/ml to 0.078 µg/ml. For active compounds the highest concentration is lowered (e.g. to 100 ng/ml), for plant extracts the highest concentration is increased to 50 µg/ml. Each drug is tested in duplicate and repeated once for active compounds showing an IC_{50} below 0.5 µg/ml. After 48 hours of incubation at 37°C, 0.5 µCi ^{3}H-hypoxanthine is added to each well. Cultures are incubated for a further 24 h before they are harvested onto glass-fiber filters and washed with distilled water. The radioactivity is counted using a BetaplateTM liquid scintillation counter (Wallac, Zurich, Switzerland). The results are recorded as counts per minute (CPM) per well at each drug concentration and expressed as percentage of the untreated controls. From the sigmoidal inhibition curves IC_{50} values are calculated.

Primary screen

K1 strain is used. The compounds are tested at 7 concentrations (5000 to 78 ng/ml). Artemisinin and chloroquine are included as reference drugs.

If the IC_{50} is >5 µg/ml, the compound is classified as inactive

If the IC_{50} is 0.5-5 µg/ml, the compound is classified as moderately active

If the IC_{50} is <0.5 µg/ml, the compound is classified as active and is further evaluated using two strains, K1 and NF54. A new range of concentrations is chosen depending on the IC_{50} determined (e.g. 100 to 1.56 ng/ml) and the assay is carried out 2x independently.

The standard drugs are chloroquine and artemisinin which are run in the same assay. The IC_{50} values for chloroquine are 2.9 ng/ml for NF54 and 48 ng/ml for K1; for artemisinin 1.9 ng/ml for NF54 and 0.8 ng/ml for K1.

Secondary screen

Test compounds are tested against a panel of say, 14 different of different origin and some show resistances to chloroquine and/or pyrimethamine. If the range of the IC_{50} values for the 14 strains is within a factor 3-5 x then the tested compound is considered not to show cross resistance.

Example B2

Malaria enzyme assays

Inhibition of *Plasmodium falciparum* dUTPase

Chemicals

2′-dUTP, was purchased from Pharmacia. $MgCl_2$, BSA, and the pH indicator cresol red were from Sigma. The buffer N, N-bis (2-hydroxyethyl) glycine (BICINE) was obtained from USB (United States Biochemical), Ohio. All the concentrations of nucleotides were calculated spectrophotometrically (HP-8453, Hewlett Packard) at 280 nm, using the extinction coefficient ($\varepsilon_{280\ nm}$=1.75 ml $mg^{-1}cm^{-1}$). Other chemicals used in these experiments were of the highest quality available.

Cloning of the *PFdut* gene

Conserved motifs of the human dUTPase enzyme were used as query to identify the *PFdut* gene in the www. tigr.org database of the *Plasmodium falciparum* 3D7 strain. The entire coding sequence was amplified by the PCR using as template cDNA and as primers the oligonucleotides *ATG-PFdut* (CATATGCATTTAAAAATTGTATGTCTG) and *TGA-PFdut* (GGATCCTCAATATTTATTATCGATGTCGATC) which were designed so that *Nde*I and *BamH*I restriction sites were introduced at the 5′ and 3′ ends for convenient cloning in the expression vector pET11 (*Stratagene*). The amplified product was cloned into pGEMT (*Promega*) and propagated in *E. coli* XL1B cells. In order to confirm the correct

sequence after amplification, sequencing was performed using an Applied Biosystems Automated Sequencer, at the Analytical Services of the Instituto de Parasitología y Biomedicina "López Neyra". These Services also supplied the oligonucleotides designed for the sequencing

P. falciparum dUTPase overexpression and purification

Recombinant *P. falciparum* dUTPase was purified from *E. coli* BL21 (DE3) cells transformed with pET-*PFdut*. Pellets from a liter of culture were resuspended in a solution consisting of buffer A (20 mM MES pH 5.5, 50 mM NaCl, 1 mM DTT) plus the protease inhibitors 1 mM PMSF, 20 μg/ml leupeptin and 1 mM benzamidine. Purification was carried out in a cold room (4°C). The soluble crude extract was obtained by sonication in a Vibra-cell (Sonics and Materials Inc. Danbury, Connecticut, USA) and centrifugation at 14000x*g*. The extract was loaded onto a phosphocellulose column (Whatman) pre-equilibrated with buffer A at a flow rate of 1 ml/min. After washing the column with 100 ml of buffer A, elution was performed using a linear NaCl gradient of 50 to 1000 mM. Peak fractions with a low concentration of contaminating protein, as judged by 15% SDS-PAGE gels, were pooled and then loaded and chromatographed on a *Superdex 200* column at a flow rate of 0.5 ml/min. The column was equilibrated with buffer B (50 mM Bicine, 1 mM DTT, 10 mM $MgCl_2$). Peak fractions were pooled and concentrated to about 5 mg/ml by ultrafiltration in a Centripep tube (*Amicon*) and stored at –80°C.

Kinetic measurements

Nucleotide hydrolysis was monitored by mixing enzyme and substrate with a rapid kinetic accessory (Hi-Tech Scientific) attached to a spectrophotometer (Cary 50) and connected to a computer for data acquisition and storage. Protons, released through the hydrolysis of nucleotides, were neutralised by a pH indicator in a weak buffered medium with similar pK_a and monitored spectrophotometrically at the absorbance peak of the basic form of the indicator. Absorbance changes were kept

within 0.1 units. The indicator/buffer pair used was cresol red/BICINE (2mM/50μM, pH 8, 573 nm). The measurements were performed at 25°C, and the solutions were previously degassed. Assays contained 30 nM purified recombinant enzyme, 50 μM dUTP, 5mM $MgCl_2$ and 2.5mM DTT, 1.25mg/ml BSA and 100mM KCl. Indicator absorbance changes corresponding to complete hydrolysis of nucleotides were recorded in the computer, and the kinetic parameters V_{max} and K_{mapp} (or slope) were calculated by fitting the data to the integrated Michaelis-Menten equation (Segel, 1975).

$$[dUMP]/t = V_{max} - K_{map}/t \ln [dUTP]/([dUTP]-[dUMP])$$

Solutions of potential inhibitors were prepared at 10mg/ml and tested routinely at concentrations of 2, 10, and 50μg/μl. A wider range of concentrations was further tested when necessary for K_i determination. The different apparent K_m values attained were plotted against inhibitor concentration and K_i values were obtained according to the following equation:

$$K_{map} = \frac{K_m}{K_i} [I] + K_m$$

Example B3

Human dUTPase assay

Human recombinant dUTPase was purified from *E. coli* BL21 (DE3) cells transformed with pETHu*dut* (Dr. P.O. Nyman, Lund University). Purification was accomplished as described for the dUTPase above except that the last step in *Superdex* 200 was omitted. Likewise, conditions for enzyme assays were the same as described above except that the enzyme concentration was 50nM.

Example B4

Trypanosoma brucei whole cell assays

Parasite cultures

Three strains of *T. brucei* spp. are used in this study: (a) *Trypanosoma brucei rhodesiense* STIB 900, a clone of a population isolated in 1982 from a patient in Tanzania which is known to be susceptible to all currently used drugs; (b) *Trypanosoma brucei gambiense* STIB 930, a derivative of strain TH1/78E (031) isolated in 1978 from a patient in Ivory Coast which is known to be sensitive to all drugs used, and (c) *Trypanosoma brucei brucei* STIB 950, a clone of a population isolated in 1985 from a bovine in Somalia which shows drug resistance to diminazene, isometamidium and quinapyramine.

The bloodstream form trypomastigotes of the strains a and c are maintained in MEM medium with Earle's salts supplemented with 25 mM HEPES, 1g/l additional glucose, 1% MEM non-essential amino acids (100x), 0.2 mM 2-mercaptoethanol, 2mM Na-pyruvate, 0.1mM hypoxanthine and 15% heat inactivated horse serum.

The bloodstream form trypomastigotes of strain b are maintained in MEM medium with Earle's salts supplemented with 25 mM HEPES, 1g/l additional glucose, 1% MEM non-essential aminoacids (100x), 0.2 mM 2-mercaptoethanol, 2mM Na-pyruvate, 0.1mM hypoxanthine, 0.05mM bathocuproine disulphonic acid, 0.15mM L-cysteine and 15% heat inactivated pooled human serum.

All cultures and assays are conducted at 37°C under an atmosphere of 5% $C0_2$ in air.

Drug sensitivity assays

Stock drug solutions are prepared in 100% DMSO (unless otherwise suggested by the supplier) at 10 mg/ml, and heated or sonicated if necessary. After use the stocks are kept at –20°C. For the assays, the compound is further diluted to the appropriate concentration using complete medium.

Assays are performed in 96-well microtiter plates, each well containing 100 µl of culture medium with 8×10^3 bloodstream forms with or without a serial drug dilution. The highest concentration for the test compounds is 90 µg/ml. Seven 3-fold dilutions are used covering a range from 90 µg/ml to 0.123 µg/ml. Each drug is tested in duplicate and each assay is repeated at least once. After 72 hrs of incubation the plates are inspected under an inverted microscope to assure growth of the controls and sterile conditions.

10µl of Alamar Blue (12.5 mg resazurin dissolved in 100 ml distilled water) are now added to each well and the plates incubated for another 2 hours. Then the plates are read with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wave length of 536 nm and an emission wave length of 588 nm. Data are analysed using the microplate reader software Softmax Pro (Molecular Devices Cooperation, Sunnyvale, CA, USA).

Primary screen

The preliminary screen uses the *Trypanosoma b. rhodesiense* strain. The compounds are tested at 7 concentrations (drug concentrations ranging from 90 µg/ml to 0.123 µg/ml in 3-fold dilutions).

If the IC_{50} is >3µg/ml, the compound is classified as inactive

If the IC_{50} is 0.2-3µg/ml, the compound is classified as moderately active

If the IC_{50} is <0.2µg/ml, the compound is classified as active

The standard drug is melarsoprol which is run in the same assay; the IC_{50} for melarsoprol is 1.6 ng/ml.

Secondary screen

Active compounds (IC_{50} <0.2μg/ml) are tested against the *Trypanosoma brucei gambiense* STIB 930 and the drug resistant *T. b. brucei* STIB 950 following the same protocol as described above.

The standard drug is melarsoprol which is run in the same assay; the IC_{50} for melarsoprol is 4.2 ng/ml for STIB 930 and 2.8 ng/ml for STIB 950

Example B5

Trypanosoma enzyme assays

Trypanosoma cruzi cell cultures:

The *Trypanosoma cruzi* Tulahuen C2C4 strain, containing the □-galactosidase (Lac Z) gene, is used. The plasmid construct by Dr. S. Reed was obtained from Dr. F. Buckner, Seattle, as epimastigotes in LIT medium.

The infective amastigote and trypomastigote stages are cultivated in L-6 cells (rat skeletal myoblast cell line) in RPMI 1640 supplemented with 2 mM L-glutamine and 10% heat-inactivated foetal bovine serum in 12.5 cm^2 tissue culture flasks. Amastigotes develop intracellularly, differentiate into trypomastigotes and leave the host cell. These trypomastigotes infect new L-6 cells and are the stages used to initiate an infection in the assay. All cultures and assays are conducted at 37°C under an atmosphere of 5% $C0_2$ in air.

Drug sensitivity assays

Stock drug solutions are prepared in 100% DMSO (dimethylsulfoxide) unless otherwise suggested by the supplier at 10 mg/ml, and heated or sonicated if necessary. The stocks are kept at –20°C. For the assays, the compound is further diluted to the appropriate concentration using complete medium.

Assays are performed in sterile 96-well microtiter plates, each well containing 100 µl medium with $2x10^3$ L-6 cells. After 24 hours 50 µl of a trypanosome suspension containing 5×10^3 trypomastigote bloodstream forms from culture are added to the wells. 48 hours later the medium is removed from the wells and replaced by 100 µl fresh medium with or without a serial drug dilution. At this point the L-6 cells should be infected with amastigotes and no free trypomastigotes should be in the medium. Seven 3-fold dilutions are used covering a range from 90 µg/ml to 0.123 µg/ml. Each drug is tested in duplicate. After 96hours of incubation the plates are inspected under an inverted microscope to assure growth of the controls and sterility.

Then the substrate CPRG/ Nonidet (50 □l) is added to all wells. A colour reaction will become visible within 2-6 hours and can be read photometrically at 540nm. Data are transfered into a graphic programme (e.g. EXCEL), sigmoidal inhibition curves determined and IC_{50} values calculated.

Primary screen

Benznidazole is used as the reference drug and shows an IC_{50} value of 0.34 µg/ml.

If the IC_{50} is > 30 µg/ml, the compound is classified as inactive.
If the IC_{50} is between 2 and 30µg/ml , the compound is classified as moderately active.
If the IC_{50} is < 2 µg/ml, the compound is classified as active.

Example B6

Leishmaniasis: macrophage *in vitro* screening model

Parasite and cell cultures

The *Leishmania.donovani* strain MHOM/ET/67/L82 obtained from Dr. S. Croft, London) is used. The strain is maintained in the Syrian Golden hamster. Amastigotes are collected from the spleen of an infected hamster Amastigotes are grown in axenic culture at 37°C in SM medium (Cunningham I., J. Protozool. 24, 325-329, 1977) at pH 5.4 supplemented with 10% heat-inactivated foetal bovine serum under an atmosphere of 5% $C0_2$ in air.

Primary peritoneal macrophages from NMRI mice are collected 1 day after a macrophage production stimulation with an i.p injection of 2ml of a 2% potato starch suspension (FLUKA, Switzerland) All cultures and assays are done at 37°C under an atmosphere of 5% $C0_2$ in air.

Drug sensitivity assays

Stock drug solutions are prepared in 100% DMSO (unless otherwise suggested by the supplier) at 10 mg/ml, and heated or sonicated if necessary. After use the stocks are kept at –20°C. For the assays, the compound is further diluted in serum-free culture medium and finally to the appropriate concentration in complete medium.

Assays are performed in sterile 16-well chamber slides (LabTek, Nalgene/Nunc Int.) To each well 100 µl of a murine macrophage suspension (4 x 10^5/ml) in RPMI 1640 (containing bicarbonate and HEPES) supplemented with 10% heat inactivated fetal bovine serum is added. After 24 hrs 100 µl of a suspension containing amastigotes (1.2 x 10^6/ml) is added resulting in a 3:1 ratio of amastigotes/macrophages. The amastigotes are harvested from an axenic amastigote culture and

suspended in RPMI/FBS. 24 hrs later, the medium containing free amastigotes is removed, washed 1x and replaced by fresh medium containing four 3-fold drug dilutions. In this way 4 compounds can be tested on one 16-well tissue culture slide. Untreated wells serve as controls. Parasite growth in the presence of the drug is compared to control wells. After 4 days of incubation the culture medium is removed and the slides fixed with methanol for 10 min followed by staining with a 10% Giemsa solution. Infected and non-infected macrophages are counted for the control cultures and the ones exposed to the serial drug dilutions. The infection rates are determined. The results are expressed as % reduction in parasite burden compared to control wells, and the IC_{50} calculated by linear regression analysis.

Primary screen

The compounds are tested in duplicate at 4 concentrations ranging from 9 to 0.3 µg/m. If the IC50 is below 0.3 µg/ml then the range is changed to 1 to 0.03 µg/ml. Miltefosine is used as the reference drug and shows an IC_{50} value of 0.325 µg/ml (0.22 – 0.42 µg/ml; n=4)

If the IC_{50} is higher than 10 µg/ml, the compound is classified as inactive.

If the IC_{50} is between 2 and 10µg/ml , the compound is classified as moderately active.

If the IC_{50} is < 2 µg/ml, the compound is classified as active and is further evaluated in a secondary screening.

Drug sensitivity assays

Stock drug solutions are prepared in 100% DMSO (dimethylsulfoxide) unless otherwise suggested by the supplier at 10 mg/ml, and heated or sonicated if necessary. The stocks are kept at –20°C. For the assays, the compound is further diluted to the appropriate concentration using complete medium.

Assays are performed in sterile 96-well microtiter plates, each well containing 100 µl medium with $2x10^3$ L-6 cells. After 24 hours 50 µl of a trypanosome suspension containing 5×10^3 trypomastigote bloodstream forms from culture are added to the wells. 48 hours later the medium is removed from the wells and replaced by 100 µl fresh medium with or without a serial drug dilution. At this point the L-6 cells should be infected with amastigotes and no free trypomastigotes should be in the medium. Seven 3-fold dilutions are used covering a range from 90 µg/ml to 0.123 µg/ml. Each drug is tested in duplicate. After 96hours of incubation the plates are inspected under an inverted microscope to assure growth of the controls and sterility.

Then the substrate CPRG/ Nonidet (50 □l) is added to all wells. A colour reaction will become visible within 2-6 hours and can be read photometrically at 540nm. Data are transfered into a graphic programme (e.g. EXCEL), sigmoidal inhibition curves determined and IC_{50} values calculated.

Primary screen

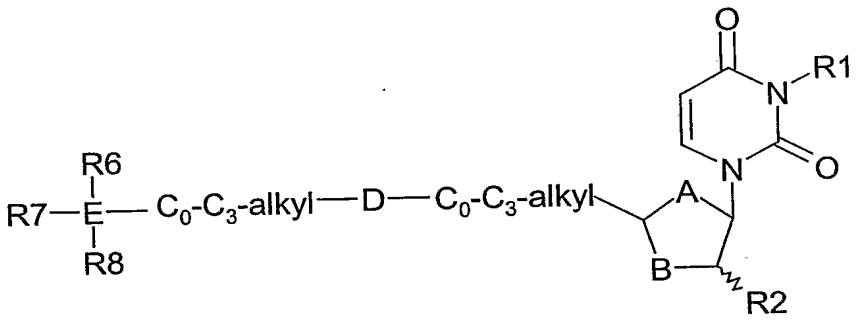
Benznidazole is used as the reference drug and shows an IC_{50} value of 0.34 µg/ml.

If the IC_{50} is > 30 µg/ml, the compound is classified as inactive.
If the IC_{50} is between 2 and 30µg/ml , the compound is classified as moderately active.
If the IC_{50} is < 2 µg/ml, the compound is classified as active.

Compounds of the invention, such as those in the examples above typically show activities in the low micromolar range for Plasmodium falciparum enzyme and cell culture, with selectivity over the human enzyme of at least 10-fold.

CLAIMS

1. A compound of the formula I



I

where

A is O, S or CH_2;

B is O, S or CHR^3;

R^1 is H, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl or a 5 or 6 membered, saturated or unsaturated ring containing 0 to 3 heteroatoms selected from N, O and S, any of which is optionally substituted with R^4;

R^2 is H, F;

R^3 is H, F, OH, NH_2 or a pharmaceutically acceptable ester, amide or ether thereof; or

R^2 and R^3 together form a chemical bond;

D is –NHCO-, -CONH-, -O-, -C(=O)-, -CH=CH, -C≡C-, -NR^5-;

R^4 is hydrogen, halo, cyano, amino, nitro, carboxy, carbamoyl, hydroxy, oxo, C_1-C_5 alkyl, C_1-C_5 haloalkyl, C_1-C_5 alkyloxy, C_1-C_5 alkanoyl, C_1-C_5 alkanoyloxy, C_1-C_5 alkylthio, -N(C_0-C_3-alkyl)$_2$, hydroxymethyl, aminomethyl, carboxymethyl; -SO_2N(C_0-C_3-alkyl), -SO_2C_1-C_5-alkyl;

R^5 is H, C_1-C_4-alkyl, C_1-C_4-alkanoyl;

E is Si or C;

R^6, R^7 and R^8 are independently selected from C_1-C_8 alkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, or a stable monocyclic, bicyclic or tricyclic ring system which is saturated or unsaturated in which each ring has 0 to 3 heteroatoms

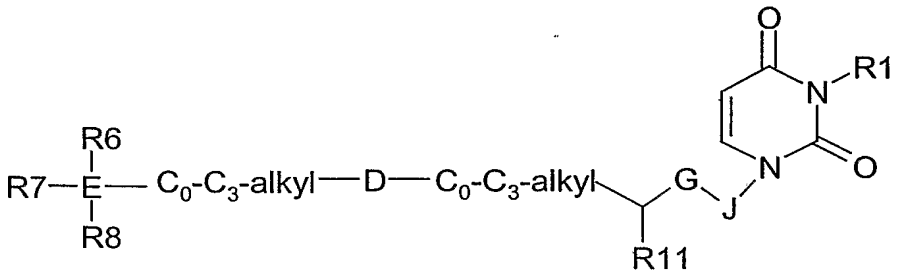
selected from N, O and S, wherein any of which R^6, R^7 and R^8 groups are optionally substituted with R^4;
with the proviso that if R^3 is H, OH, F, NH_2 or a bond, then at least one of R^6, R^7 and/or R^8 comprises an unsaturated ring; and the further proviso that the compound is not 5'-O-(4-4'-dimethoxytrityl)-2'-deoxyuridine:
and pharmaceutically acceptable salts thereof.

2. A compound according to claim 1, wherein A is –O- and B is –CHR^3-, or A is –O and B is –S-.

3. A compound according to claim 1, wherein R^2 and R^3 form a chemical bond.

4. A compound according to claim 1, wherein R^3 is OH or F.

5. A compound of the formula II:



II

R^1 is H, C_1-C_5 alkyl, C_2-C_5 alkenyl, C_2-C_5 alkynyl or a 5 or 6 membered, saturated or unsaturated ring containing 0 to 3 heteroatoms selected from N, O and S, any of which is optionally substituted with R^4;
D is –NHCO-, -CONH-, -O-, -C(=O)-, -CH=CH, -C≡C-, -NR^5-;
R^4 is hydrogen, halo, cyano, amino, nitro, carboxy, carbamoyl, hydroxy, oxo, C_1-C_5 alkyl, C_1-C_5 haloalkyl, C_1-C_5 alkyloxy, C_1-C_5 alkanoyl, C_1-C_5 alkanoyloxy, C_1-C_5 alkylthio, -N(C_0-C_3-alkyl)$_2$, hydroxymethyl, aminomethyl, carboxymethyl; -SO_2N(C_0-C_3-alkyl), -SO_2C_1-C_5-alkyl;
R^5 is H, C_1-C_4-alkyl, C_1-C_4-alkanoyl;

E is Si or C;

R^6, R^7 and R^8 are independently selected from C_1-C_8 alkyl, C_2-C_8 alkenyl, C_2-C_8 alkynyl, or a stable monocyclic, bicyclic or tricyclic ring system which is saturated or unsaturated in which wherein each ring has 0 to 3 heteroatoms selected from N, O and S, and wherein any of which R^6, R^7 and R^8 groups are optionally substituted with R^4;

G is –O-, -S-, -CHR^{10}-, -C(=O)-;

J is -CH_2-, or when G is CHR^{10} may also be –O- or –NH-;

R^{10} is H, F, -CH_3, -CH_2NH_2, -CH_2OH, -OH;

R^{11} is H, F, -CH_3, -CH_2NH_2, -CH_2OH, $CH(OH)CH_3$, $CH(NH_2)CH_3$ or a pharmaceutically acceptable ether, amide or ester thereof; or

R^{10} and R^{11} together define an olefinic bond, or together form a –CH_2- group, thereby defining a *cis* or *trans* cyclopropyl group;

with the proviso that if R^{11} is H, CH_3, CH_2NH_2, CH_2OH, a bond or –CH_2-, then at least one of R^6, R^7 and/or R^8 comprises an unsaturated ring:

and pharmaceutically acceptable salts thereof.

6. A compound according to claim 5, wherein G is –O- or -CH_2-.

7. A compound according to claim 5, wherein R^{10} and R^{11} define an olefinic bond or a cyclopropyl group.

8. A compound according to claim 5, wherein R^{11} is H or CH_2OH.

9. A compound according to claim 1 or 5, wherein R^1 is H.

10. A compound according to claim 1 or 5, wherein C_0-C_3-alkyl-D-C_0-C_3-alkyl is oxymethyl, oxyethyl or oxypropyl.

11. A compound according to claim 1 or 5, wherein C_0-C_3-alkyl-D-C_0-C_3-alkyl is aminomethyl, aminoethyl or aminopropyl.

12. A compound according to claim 5, wherein D-C_0-C_3-alkyl is –O- or –NH-.

13. A compound according to claim 1 or 5, wherein R^6 is optionally substituted phenyl.

14. A compound according to claim 13 wherein R^8 is optionally substituted phenyl.

15. A pharmaceutical composition comprising a compound as defined in any preceding claim and a pharmaceutically acceptable carrier or diluent therefore.

16. Use of a compound as defined in any of claims 1 to14 in the manufacture of a medicament for the treatment of parasitic infections in mammals, including man.

17. Use according to claim 16 wherein the parasite is a falciparum species.

18. Use of a compound as defined in claim 1, but including 5'-O-(4-4'-dimethosytrityl)-2'-deoxyuridine, in the manufacture of a medicament for the treatment or prophylaxis of malaria.